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## Arkansas Animal Science Department Report 2013

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# *Arkansas* **Animal Science** **Department Report • 2013**



David L. Kreider, Editor • Paul Beck, Assistant Editor

**UofA**

DIVISION OF AGRICULTURE  
RESEARCH & EXTENSION

*University of Arkansas System*

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ARKANSAS AGRICULTURAL EXPERIMENT STATION

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**ARKANSAS ANIMAL SCIENCE  
DEPARTMENT REPORT 2013**

*Edited by*

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*Assistant Editor*

**Paul Beck**

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University of Arkansas*

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## INTRODUCTION

Welcome from the Department of Animal Science! This is the 16th 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 Drs. David Kreider and Paul Beck who served as co-editors. With an ever-increasing human population, a demand for increased food production will be needed in the next few years. As you read through the following research projects, I am sure you will agree research generated from this department will help in developing those best management practices that will increase whole farm/ranch efficiency, and ultimately, increase producer profitability.

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 [animalscience.uark.edu](http://animalscience.uark.edu), the Livestock and Forestry Research Station website [Batesvillestation.uark.edu](http://Batesvillestation.uark.edu), the Southwest Research and Extension Center website [swrec.uark.edu](http://swrec.uark.edu), and the Southeast Research and Extension Center website [aes.uark.edu/serec](http://aes.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. The department is blessed to have a very supportive community of agriculture stakeholders in the state that are, and will continue to be, instrumental in our growth and advancement.

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,

A handwritten signature in black ink that reads "Michael Looper". The signature is written in a cursive style with a long horizontal flourish at the end.

Michael Looper  
Department Head



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## 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.,  $9.1 \pm 1.2$ ). 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 posi-

tive 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  $+1$ .





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## 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 <sup>2</sup>	simple coefficient of determination
R <sup>2</sup>	multiple coefficient of determination
s	Second(s)
SD	standard deviation
SE	standard error
SEM	standard error of the mean
TDN	total digestible nutrients
wk	week(s)
wt	Weight
yr	year(s)

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## TABLE OF CONTENTS

### *Beef Production and Management*

<b>Effects of lactate dehydrogenase B haplotypes on beef cow production</b> O. Alaamari, M. Sales, M. Looper, and C. Rosenkrans, Jr.....	11
<b>Effects of castration with or without analgesia on growth performance, hematology, and behavior in neonatal beef cattle</b> A.C. Brown, J.G. Powell, M.S. Gadberry, E.B. Kegley, J.T. Richeson, J.A. Hornsby, J.L. Reynolds, B.R. Lindsey, R.W. Shofner and Y.V. Thaxton.....	14
<b>Intake, digestibility and in situ disappearance of bermudagrass hay diets supplemented with different types of distillers' grains for lactating cows</b> K.P. Coffey, A.N. Young, E.B. Kegley, J.A. Hornsby, J. Hollenback, and D. Philipp.....	19
<b>Isoflupredone acetate as ancillary therapy for bovine respiratory disease in high-risk stocker calves</b> C.E. Crews, J.G. Powell, E.B. Kegley, J.L. Reynolds, and J.A. Hornsby.....	22
<b>Estrous response and pregnancy rates in beef cows following a 6 or 7 day controlled internal drug release synchronization protocol</b> A.J. Davis, R.W. Rorie, J.G. Powell, T.D. Lester, E.A. Backes, and B.R. Lindsey.....	25
<b>Influence of growth-promoting implants on development of low weight replacement beef heifers</b> T.L. Devine, C.F. Rosenkrans, Jr., D. Philipp, D.S. Hubbell, III, E.B. Backes, A.J. Davis, T.D. Lester, R.W. Rorie, C.L. Orr, R. Rhein, and M.L. Looper .....	27
<b>Response of Angus cows and their suckling calves to an injectable trace mineral supplement</b> M.S. Gadberry and B. Baldrige.....	30
<b>Effects of copper oxide bolus administration on productivity and copper status in grazing beef calves supplemented with dried distillers' grains</b> J. Hawley, E.B. Kegley, J.M. Bauer, and J.G. Powell .....	33
<b>Effects of feed delivery methods for stocker calves grazing bermudagrass on growth performance, behavior, and labor inputs</b> E. Mashie, E.B. Kegley, S. Gadberry, A.K. Sexten, J. Powell, J.L. Reynolds, and J.A. Hornsby.....	36
<b>Horn fly and sire breed affects on milk production traits of beef cows</b> A.R. Mays, M.A. Brown and C.F. Rosenkrans, Jr. ....	41
<b>Association of genotypes from the promoter region of the bovine prolactin gene with milk production traits and horn fly resistance of beef cows</b> A.R. Mays, M.A. Brown and C.F. Rosenkrans, Jr. ....	47
<b>Effects of ergot alkaloids on bovine sperm motility</b> R.J. Page, R.W. Rorie, T.D. Lester, C. Williams, M. Rowe, and C.F. Rosenkrans, Jr.....	50
<b>Clover emergence and biomass production in wooded areas</b> D. Philipp, J. Jennings, D. Burner, B. McPeake, D. Pote, B. Woolley, and R. Rhein.....	55
<b>Effect of hormonal growth implant administration timing on health, performance, and immunity of beef stocker cattle</b> J. Richeson, P. Beck, D. Hubbell, S. Gadberry, B. Kegley, J. Powell, and F. Prouty.....	58
<b>Effect of timing of insemination with sorted semen on subsequent pregnancy rate in postpartum beef cows synchronized with a modified 14-day progesterone protocol</b> R.W. Rorie, J.G. Powell, T.D. Lester, A.J. Davis, and B.R. Lindsey .....	64
<b>Intake, digestibility and ruminal fermentation characteristics of cows limit-fed co-product commodity feeds</b> W.B. Smith, K.P. Coffey, R.T. Rhein, E.B. Kegley, D. Philipp, J.D. Caldwell and A.N. Young.....	67

<b>Production characteristics and blood metabolites of gestating cows limit-fed soybean hulls</b> W.B. Smith, K.P. Coffey, J.D. Tucker, D.S. Hubbell III , E.B. Kegley, D. Philipp, J.D. Caldwell and A.N. Young .....	71
<b>Balking behavior in cattle breed-type prevalence based on coat color and potential carcass implications</b> M.L.Thomas, Y.V. Thaxton, A.H. Brown Jr., K.E. Pfalzgraf, K.S. Anschutz, and C.F. Rosenkrans Jr. ....	74
<b>Population and price differences for Arkansas sale barn marketed calves from 2000 to 2010 due to management influenced phenotype</b> T.R. Troxel and M.S. Gadberry .....	77
<b>Population and price differences for Arkansas sale barn marketed calves from 2000 to 2010 due to genetically influenced phenotype</b> T.R. Troxel and M.S. Gadberry .....	81
<b>Relationships of polymorphisms of lactate dehydrogenase to heifer immune response</b> C.M. Turner, A.H. Brown Jr., J.G. Powell, F.W. Pohlman, K.S. Anschutz, J.A. Hornsby, J.L. Reynolds, and C.F. Rosenkrans, Jr.....	88
<b>Evaluation of hair shedding scores in relation to maternal traits and productivity in beef cattle</b> A.L. Williams, A.H. Brown, Jr., J.G. Powell, C.M. Turner, K.S. Anschutz, B.R. Lindsey, R.W. Shofner, and C.F. Rosenkrans, Jr. ....	93
 <i>Forages and Forage Management</i>	
<b>Performance by yearling Katahdin ewes grazing tall fescue pastures using continuous or rotational grazing schemes—2 year summary</b> E.A. Backes, J.D. Caldwell, B.C. Shanks, K.R. Ness, A.N.V. Stewart, L.S. Wilbers, C.A. Clifford-Rathert, A.K. Wurst, H.A. Swartz, D.L. Kreider, and M.L. Looper .....	99
<b>Intake and digestibility of heat-damaged hay by Katahdin ewes</b> W.B. Smith, K.P. Coffey, E.B. Kegley, J.D. Caldwell, A.N. Young, E.A. Backes, J. Kanani and D. Philipp.....	101
 <i>Meats and Food Safety</i>	
<b>Instrumental color properties of ground beef processed from beef trimmings treated with peroxyacetic acid and/or organic acids</b> P.N. Dias-Morse, F.W. Pohlman, S.D. Pinidiya and C.L. Coffman .....	105
<b>Microbial characteristics of ground beef processed from beef trimmings decontaminated by peroxyacetic acid alone or followed by organic acids interventions</b> P.N. Dias-Morse, F.W. Pohlman, S.D. Pinidiya, and C.L. Coffman .....	110
<b>Beef quality attributes of precooked ground beef patties formulated with mature bull trimmings</b> J.J. Hollenbeck, J.K. Apple, J.W.S. Yancey, K.N. Kerns, and A.N. Young.....	115
<b>Effects of lactic acid enhancement on beef quality attributes of mature bull strip loins</b> J.J. Hollenbeck, J.K. Apple, J.W.S. Yancey, A.N. Young, C.T. Moon, T.M. Johnson, and D.L. Galloway .....	120



# Effects of lactate dehydrogenase B haplotypes on beef cow production

O. Alaamari<sup>1</sup>, M. Sales<sup>1</sup>, M. Looper<sup>1</sup>, and C. Rosenkrans, Jr.<sup>1</sup>

## Story in Brief

Lactate dehydrogenase (LDH) catalyzes the conversion of the pyruvate to lactate (forward) or lactate to pyruvate (reverse). Our objectives were to evaluate the association between LDH-B mutations and beef cow productivity. Four single nucleotide polymorphisms (SNP) were detected, and eight haplotypes were assigned based on SNPs: G-348A, A-261G, N-222D, and C541A. Specific primers were designed for polymerase chain reaction and amplification of 452-base pair fragment and 457-base pair fragments of the bovine LDH-B coding sequence and promoter, respectively. Brahman-influenced cows (n = 109) were managed to achieve either low (BCS = 4.3 ± 0.1) or high (BCS = 6.4 ± 0.1) body condition. Cows grazed stockpiled and spring growth endophyte-infected tall fescue pastures prior to breeding season, and grazed bermudagrass during the 60-d breeding period. At base position G-348A, cows that were heterozygous (GA) had a lower calving rate than cows that were homozygous with the primary allele (53.3% vs. 79.1%, respectively). Our results suggest that mutations associated with LDH-B gene may be used as a genetic marker for the selection of cows with improved fertility.

## Introduction

Lactate dehydrogenase can be found in wide variety of organisms, including mammals, and consists of at least two structural-function domains. In mammals and birds, lactate dehydrogenase is present in five somatic isoenzymes in which activity and regulation can be organ-specific. These five somatic isoenzymes are tetrameric combinations of two 35-kDa subunits, including A (muscle) and B (heart; Cardinet, 1997). Lactate dehydrogenase (LDH) as a cytoplasmic enzyme catalyzes the conversion of pyruvate to lactate (forward; LDHf) in tissues with low oxygen concentrations or lactate to pyruvate (reverse; LDHr) in the presence of normal oxygen concentrations. Looper et al. (2002) suggested that cattle reproduction was related to decreased LDHr activity in heifers. Our objective was to determine the effects of LDH-B mutations and reproductive performance of Brahman-influenced cows.

## Materials and Methods

**Animal Management.** Spring-calving crossbred (1/4 to 3/8) multiparous Brahman-influenced cows (n = 109) were managed to achieve low or moderate body condition (BC) at parturition. Cows grazed (~162 d) stockpiled and spring growth, endophyte-infected tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire) pastures prior to the breeding season. To obtain desired BC, stocking rate was either 1 cow/0.8 acres (low BC; n = 50) or 1 cow/2 acres (moderate BC; n = 49).

**Blood Collection.** Blood samples were collected from cows at 35 d before the breeding season. Plasma and buffy coats were harvested within 8 h of blood collection. Blood samples were maintained at 39.2 °F until centrifuged (1,500 × g for 25 min). Following centrifugation, buffy coats were stored at -112 °F until genomic DNA was harvested using a commercially available kit (Qiagen).

**Polymerase Chain Reaction (PCR).** A Peltier thermal cycler 100 (MJ Research, Waltham, Mass.) was used for amplification. The thermal cycler began with a denaturation temperature of 201 °F for 2 min and then cycled at 201 °F for 30 s, 131 °F for 1 min and 154 °F for 1 min. After cycling 35 times, a final extension occurred at 154 °F for 10 min. Samples were held at 46 °F until sequenced.

**Primers.** Two primers were designed to amplify a 452-base pair (bp) fragment (bases 489 to 940 of accession number aj401268) of the bovine LDH-B coding sequence (cds) (5'-GTACAGTCCTG CCTGCATCA -3' and 5'-CCATTGTTGACACTGGGTGA -3'). Two additional primers were designed to amplify a 457-bp fragment from position -269 to 30 of the bovine LDH-B promoter (accession number NW001495085; 5'-ACACACCAGCAGCATCTCAG-3' and 5'-GATAAGGGCTGCACGAAGAC-3'). Amplification products were sequenced by the DNA Core Lab using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif.). Sequence identity was compared using the web-based software package ClustalW (European Bioinformatics Institute, Cambridge, UK). Based on SNP sites, cows were genotyped and haplotypes assigned. Associations between genotypes or haplotypes and calving percentage were tested by Chi-square.

## Results and Discussion

Low BC cows (BW = 931.0 ± 34.8 lb) weighed less than moderate cows (BW = 1,168.9 ± 35.3 lb); furthermore low BC cows (4.3 ± 0.1) were of poorer condition when compared with moderate cows (6.1 ± 0.1). Body condition was assessed on the 1 to 9 scale (1 = emaciated to 9 = obese).

**Identification of Polymorphisms.** Three single nucleotide polymorphism (SNP) sites were identified in the promoter region and one SNP in the coding sequence of LDH-B gene. Genotype distribution for SNP (G-348A, A-261G, N-222D, and C541A) are presented in Table 1.

**Base Position -348.** A transition from a guanine to adenine (G to A) was detected. Twenty cows were either homozygous or heterozygous with the minor allele (Table 1). Cows that were heterozygous (GA) had a lower calving rate than cows that were homozygous with primary allele (53.3% vs. 79.1%, respectively; Fig. 1).

**Base Position-261.** A base transition (adenine to guanine (A to G)) was identified. Genotype at A-261G did not influence ( $P > 0.10$ ) the calving rate. Twenty-five cows were either homozygous or heterozygous with the minor allele (Table 1).

**Base Position -222.** Deletion of six nucleotides (GGCCGC) was detected starting at base -222. Fifty-five cows were either hetero or

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homozygous for the deletion (Table 1). Calving percent was not influenced ( $P > 0.10$ ) by the SNP (N-222D).

**Base Position 541.** Transversion was detected from a cytosine to adenine (C to A). Sixty-five cows were either hetero or homozygous for the minor allele (Table 1). Calving rate was not influenced ( $P > 0.10$ ) by the SNP.

**LDH-B Haplotypes.** Eight unique haplotypes were derived from the four SNP sites (Table 2). Number of cows per haplotypes ranged from two to thirty-three. Haplotype 1 had the same sequence as that published at GenBank (GenBank accession numbers aj401268 and NW001495085).

Circulating lactate dehydrogenase activity has been associated with beef cattle growth, development, and reproductive capacity. Results presented in this article suggest a genetic linkage between calving percent and LDH-B genotypes. Linking genetic mutations to enzymatic activity and profitability traits will be the subject of future research.

## Implications

The SNPs that were identified in the promoter and coding sequence region of the bovine LDH-B gene may be used as genetic markers for selecting Brahman-influenced cows which have greater calving rate which should result in greater profitability.

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- Looper, M.L., T.P. Neidecker, C.W. Wall, S.T. Reiter, R. Flores, A.H. Brown Jr., Z.B. Johnson, and C.F. Rosenkrans Jr. (2002). Relationship of Lactate Dehydrogenase Activity with Body Measurements of Angus  $\times$  Charolais Cows and Calves. *Prof. Anim. Sci.* 18:120.

**Table 1. Description of SNP in amplicons (452-bp and 457-bp) of bovine LDH-B coding sequence and promoter, respectively.**

Polymorphism <sup>†</sup>	Genotype Distribution <sup>‡</sup>			MAF <sup>§</sup>
	Homo	Hetero	homo	
G-348A	102	17	3	9.4
A-261G	97	21	4	11.9
N-222D	67	49	6	25
C541A	68	63	2	25.2

<sup>†</sup> Single nucleotide polymorphism (SNP) occurred at the number indicated. First letter the primary allele and the letter following the digits is the minor allele (D represent deletion of six nucleotides).

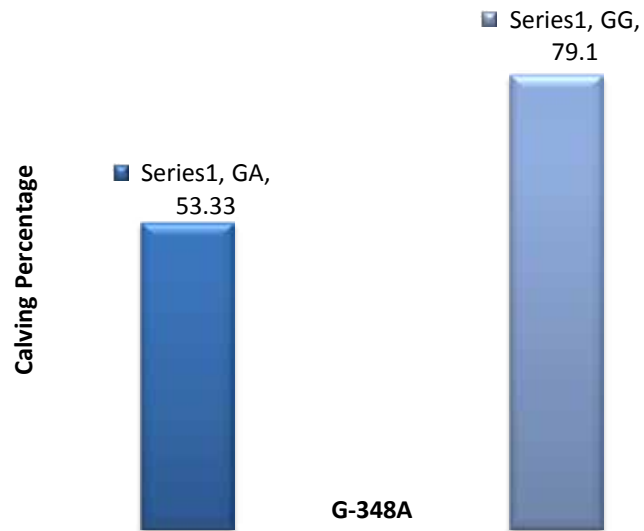
<sup>‡</sup> Number of cows that were homozygous for the major allele (Homo), heterozygous (Hetero), and homozygous for the minor allele.

<sup>§</sup> Minor allele frequency expressed as a percent.

**Table 2. Haplotype frequency in amplicons (452-bp and 457-bp) of bovine LDH-B coding sequence and promoter, respectively.**

Number	Haplotype <sup>†</sup> Sequence	Number of observations
1	GANC	33
2	AADA	10
3	AADC	10
4	GADA	8
5	GADC	2
6	GAN A	25
7	GGDA	12
8	GGDC	9

<sup>†</sup> All of these haplotypes have the same order of single nucleotide polymorphisms (SNP): G-348A, A-261G, N-222D, and C541A. Haplotype one represents the published sequence (GenBank accession numbers aj401268 and NW001495085). Deletion of six nucleotides is represented as D.



**Fig. 1. Percentage of cows calving by LDH-B promoter single nucleotide polymorphism G-348A. The genotype distribution was 102 and 17 for GG (homozygous) and GA (heterozygous), respectively.**



# Effects of castration with or without analgesia on growth performance, hematology, and behavior in neonatal beef cattle

A.C. Brown<sup>1</sup>, J.G. Powell<sup>1</sup>, M.S. Gadberry<sup>2</sup>, E.B. Kegley<sup>1</sup>, J.T. Richeson<sup>3</sup>, J.A. Hornsby<sup>1</sup>,  
J.L. Reynolds<sup>1</sup>, B.R. Lindsey<sup>1</sup>, R.W. Shofner<sup>1</sup> and Y.V. Thaxton<sup>4</sup>

## Story in Brief

Angus-sired (n = 30) and Hereford-sired (n = 32) bull calves were assigned randomly to 1 of 3 treatments. Treatments were 1) surgical castration near birth, 2) oral administration of the analgesic meloxicam (1 mg/kg of body weight) followed by surgical castration near birth, or 3) bulls remained intact. Calf standing and lying activity was monitored by recording x and y axis positions of a datalogger accelerometer attached to the metatarsus of the right leg for 7 d, in addition blood was collected on d 0, 1, 3, and 7. Body weight was recorded on all calves on d 4, 33, 66, 116, 162, 199, and 214. Average daily gain did not differ between treatments ( $P \geq 0.88$ ) through weaning, and no differences were recorded at any weigh date. White blood cell counts were not affected by treatment ( $P = 0.47$ ), but had a day effect on d 1 and d 3 ( $P = 0.002$ ). A treatment  $\times$  day interaction was noted for the percentage of neutrophils ( $P = 0.006$ ) and percentage of lymphocytes ( $P = 0.02$ ). The neutrophil to lymphocyte ratio showed a decrease with day ( $P < 0.0001$ ). Although means were similar ( $P = 0.50$ ), bull calves spent the least proportion of time standing, followed by calves castrated without an analgesia, and finally calves castrated with an analgesia had the greatest proportion of time spent standing. Time spent lying on sternum decreased when castrated was compared to non-castrated ( $P < 0.01$ ) and tended to be less for castrated calves given an analgesic vs. those without analgesic ( $P = 0.08$ ). Overall, bull calves subjected to neonatal castration with or without analgesia did not appear to exhibit negative diversions to the hematology, standing and lying behavior, and growth performance through weaning when compared to intact bulls.

## Introduction

There are approximately 15 million bull calves castrated each year in the United States (USDA, 2012). While castration is necessary to reduce aggressive and sexual behavior, it may cause pain and stress that can temporarily reduce performance. Fisher et al. (1996) conducted a study that resulted in a reduction of average daily gain (ADG) of beef calves that were castrated at 5.5 mo of age, compared to intact cohorts. Altered behavioral responses of castrated beef calves were noted by Sutherland et al. (2011) reporting that 3 month old castrated calves had increased frequency of tail wagging and reduced time spent eating compared to intact cohorts.

There is currently no pharmaceutical approved by the FDA for analgesia in cattle. Meloxicam, however, is an FDA approved analgesic and prescribed for pain relief in other species, such as companion animals. Coetzee et al. (2011) reported that oral administration of meloxicam 24 h before castration reduced the incidence of bovine respiratory disease in newly received beef calves.

The objective of this study was to determine the effects of castration in neonatal calves at birth with or without pain control on growth performance, hematology, and standing and lying behavior.

## Materials and Methods

Angus-sired (n = 30) and Hereford-sired bull calves (n = 32) were born between September 1, 2011 and November 18, 2011. Bulls were assigned randomly within sire group to 1 of 3 treatments within 72 h of birth. Treatment 1 included surgical castration near

birth without an analgesic, while treatment 2 included surgical castration near birth with an analgesic and treatment 3 calves were left intact until weaning. Throughout the study, all animals were housed and cared for in compliance with procedures approved by the University of Arkansas Animal Care and Use Committee, protocol number: 12003.

On d 0, body weights were recorded and surgical castration was performed for the appropriate treatments. If the calf was assigned to receive an analgesic, meloxicam (Yung Shin Pharmaceutical Ind. Co. Ltd.; Tachan Taichung, Taiwan) was administered orally at 1 mg/kg of BW immediately before castration. Meloxicam pills were crushed and mixed with 20 ml of sterile water before administering as a drench solution. The same syringe was then rinsed with another 20 ml of water, and administered to the calf. Castration was performed with a scalpel, removing the bottom third of the scrotum. The testes were pulled from the inside of the scrotum, and the spermatic cord was severed with a scalpel. The wound was sprayed with a disinfectant and an insecticide, and the calf was allowed to return to its dam.

Thirteen randomly selected Angus sired calves and their dams and 16 randomly selected Hereford sired calves and their dams, with equal representation of each treatment, were moved to a separate facility containing six 2000 ft<sup>2</sup> concrete floored pens for a 7-d period for further testing. All other calves were returned to their original pastures with their dams. Blood samples were taken from the previously selected calves, via jugular venipuncture, and were collected into K<sub>2</sub>EDTA tubes (Vacutainer, BD, Franklin Lakes, N.J.). Calf lying and standing behavior were determined by attaching a

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datalogger accelerometer (HoBeware Pendant G, Onset Computer Corp, Bourne, Mass.) lateral to the metatarsus of the right hind leg, using Velcro (Velcro USA, Inc, Manchester, N.H.) that had a heavy duty adhesive on the back of each side. The datalogger was further secured by placing Vetrap (3M, St Paul, Minn.) around the leg and over the accelerometer. The accelerometers were programmed to record at 20 s intervals, and date, time, *x* and *y* axis positions were recorded for 7 d. The average file length was  $29,654 \pm 897$  records among 28 calves; 1 calf's data was removed due to the recording time being incorrectly set to 2 min.

On d 0, 1, 3 and 7, body weight was recorded and blood was drawn from selected calves. A complete blood count was performed (Hemavet 950FS, Drew Scientific, Waterbury, Conn.) within 5 h of blood samples being collected. At the end of the 7-d period, calves were returned, with their dams, to their original pasture.

Interim body weights (using the average birth date as d 1) were recorded on d 4, d 33, d 66, d 116, 162, 199, and 214. On d 66, calves were vaccinated for clostridial and respiratory disease, and booster vaccinations as well as an anthelmintic were administered on d 199. Weaning occurred on d 214.

Results for blood counts and body weights were analyzed using the PROC MIXED procedure of SAS. Statistical significance was considered for a *P*-value of less than or equal to 0.05. Kenward Rogers test was used as the degrees of freedom selection method. The random effect was sire and the subject of the statement was calf. The number of events for each behavior was determined within day for each calf and the average duration of each event was determined. Both number of events and average duration of events were analyzed in GLIMMIX as a repeated measure. The number of events for each behavior was determined within day for each calf and the average duration of each event was determined. Both number of events and average duration of events were analyzed in GLIMMIX as a repeated measure.

## Results and Discussion

Birth weights, interim weights, and weaning weight (Table 1) were similar ( $P \geq 0.49$ ) between treatments. Average daily gain from birth to weaning was also similar between treatments ( $P = 0.88$ ). These results are similar to those of Coetzee et al. (2011), who noted that newly received stocker calves that received meloxicam 24 h before processing had no significant difference in the rate of weight gain. These results are also similar to Imler et al. (2011), who noted that calves castrated at 36 d of age had no differences in weaning weights or ADG compared to calves castrated at 131 d of age, showing that later castration did not benefit the weight gain of the animal.

White blood cell counts were not affected by treatment ( $P = 0.47$ ), but decreased on d 1 and d 3 ( $P = 0.002$ ; Fig. 1) compared to d 0. Chase et al. (1995) reported a treatment  $\times$  day interaction in white blood cell counts ( $P < 0.02$ ) in bulls castrated at approximately 21 months of age, and white blood cells counts of castrated bulls tended to rise and fall over a wider range than those of non-castrated bulls. Over the sampling period, neutrophil percentage steadily decreased and there was a treatment  $\times$  day interaction for percentage of neutrophils ( $P = 0.006$ ; Fig. 2). Over the sampling period, lymphocyte percentages steadily increased and a treatment  $\times$  day interaction was noted for the percentage of lymphocytes ( $P = 0.02$ ; Fig. 3). When the neutrophil to lymphocyte ratio was compared, the ratio decreased with day ( $P < 0.0001$ ), however, no

differences were noted in treatment ( $P = 0.32$ ), and no treatment  $\times$  day interaction was observed ( $P = 0.12$ ). These results were somewhat expected because as a calf ages to 2 d, the neutrophil count normally decreases, and by 3 wk the lymphocyte count is higher than that of the neutrophils (Smith, 2008). Our results differed from those of Ballou et al. (2011), who noted that castration of 3 month old bull calves elevated the neutrophil to lymphocyte ratio ( $P < 0.01$ ) 24 h after castration. Hematocrit was not affected by treatment ( $P = 0.29$ ). However, results indicated a day effect ( $P < 0.0001$ ) and a treatment  $\times$  day interaction ( $P = 0.03$ ) for hematocrit. On d 1, hematocrit was highest for bulls, intermediate for calves castrated with analgesia, and least for calves castrated without analgesia with treatment means of 31.1%, 29.3%, and 27.2%, respectively ( $P = 0.02$ ).

Overall proportion of time expressed in lying flat on side (C1), standing (C2) and lying on sternum (C3) was 0.36, 0.23, and 0.41, respectively. Proportion of daily activity expressed as C1 showed calves castrated with analgesia to have the greatest amount, and bulls to have the least ( $P = 0.05$ ). However, test of simple effects by day indicated no treatment differences on most days with the exception of d 5 where castration at birth with analgesia differed from castration without analgesia and non-castrated calves ( $P < 0.01$ ). Proportion of activity expressed as C2, standing, did not differ among treatments over time. These results were similar to those reported by White et al. (2008), that indicated no difference ( $P = 0.21$ ) in the proportion of time spent standing following castration when comparing a control group and castration group of mixed-breed beef calves.

Proportion of time expressed as C3, lying on sternum, was 0.38, 0.40, and 0.42 ( $\pm 0.01$ ) for castrated with an analgesic, castrated without an analgesic, and non-castrated, respectively. Proportions of time spent lying on sternum tended to be decreased for all castrated calves compared to non-castrated ( $P < 0.01$ ) and tended to be less for castrated calves given an analgesic vs. those without an analgesic ( $P = 0.08$ ).

All treatments exhibited lying on sternum approximately four times each day, with a tendency for calves castrated with analgesia to have more occurrences than bulls. There were no differences noted in the number of events of standing between treatments; and no differences in events for treatments for lying on side. The average amount of time (min) spent in each position was calculated. Differences among treatments for mean duration were observed for C3. Lying on sternum duration for calves castrated with an analgesic, castrated without an analgesic, and bulls, was 12.2 ( $\pm 1.7$ ), 16.3 ( $\pm 1.6$ ), and 17.0 ( $\pm 1.1$ ), respectively. Castrated tended to spend less time lying on sternum than non-castrated ( $P = 0.09$ ) and calves without an analgesic tended to have an increase in lying duration compared with calves receiving an analgesic ( $P = 0.09$ ).

## Implications

Overall, castration and analgesia had an effect on the lying behavior of calves when castration was performed near birth. Also, minor changes were noted in the percentages of neutrophils and lymphocytes during the first week, and an increased hematocrit was detected in bulls after the first day. The data analysis shows that castrating near birth, with or without analgesia, had no effect on calf weight or average daily gain up until weaning. Therefore, the greater market value of steers would provide reason to encourage cattle producers to castrate at birth.

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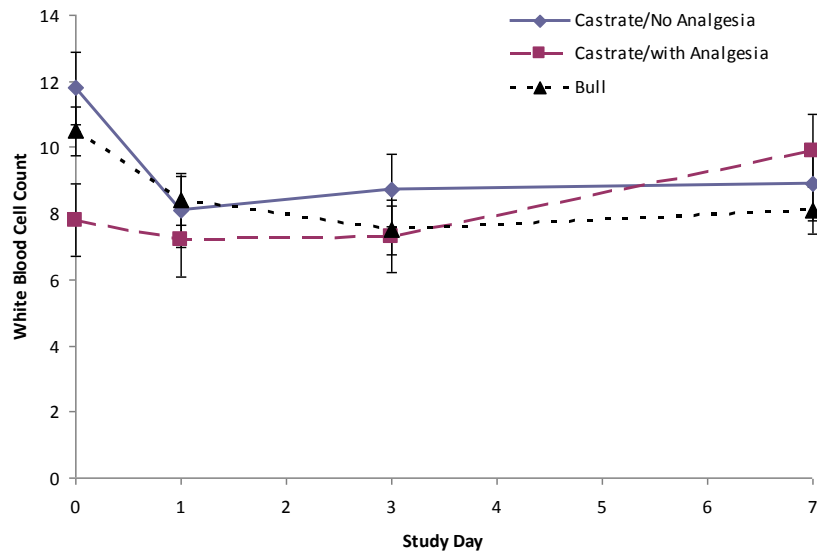
**Table 1. Effects of castration, with or without analgesia, on body weight and average daily gain (lb; Mean  $\pm$  SE).**

Time of measurement	Castration at birth without analgesia <sup>a</sup>	Castration at birth with analgesia <sup>a</sup>	Bulls <sup>a</sup>	P-value
Birth weight	73 $\pm$ 3.5	75 $\pm$ 3.3	73 $\pm$ 2.3	0.76
D 4 <sup>b</sup>	114 $\pm$ 9.9	111 $\pm$ 9.9	109 $\pm$ 6.97	0.91
D 33	156 $\pm$ 9.9	164 $\pm$ 9.9	153 $\pm$ 6.8	0.65
D 66	217 $\pm$ 15.1	209 $\pm$ 14.6	196 $\pm$ 10	0.49
D 116	297 $\pm$ 15.8	287 $\pm$ 15.2	279 $\pm$ 10.4	0.63
D 162	356 $\pm$ 18.9	351 $\pm$ 18.2	348 $\pm$ 12.5	0.94
D 199	419 $\pm$ 22.1	414 $\pm$ 21.3	421 $\pm$ 14.6	0.96
D 214	478 $\pm$ 23.7	472 $\pm$ 22.9	481 $\pm$ 15.7	0.95
ADG <sup>c</sup>	1.82 $\pm$ 0.09	1.79 $\pm$ 0.08	1.84 $\pm$ 0.06	0.88

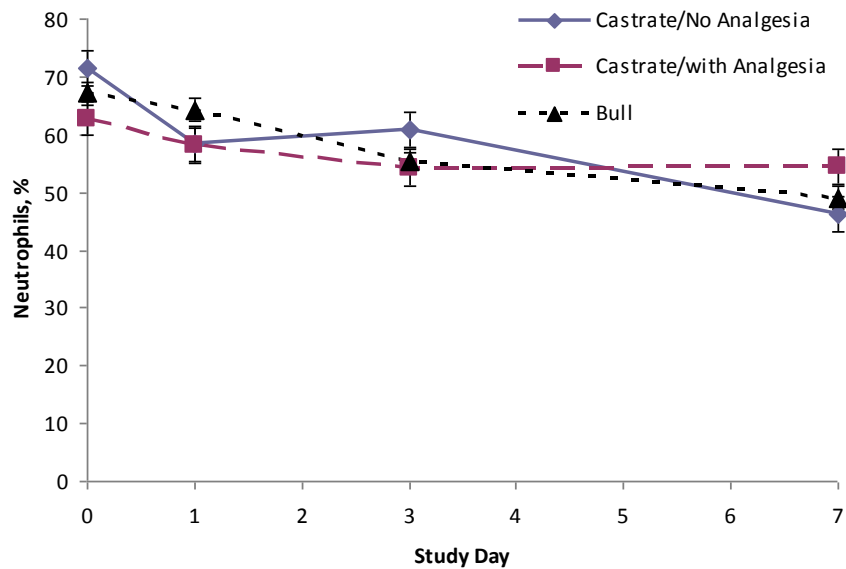
<sup>a</sup>Mean  $\pm$  SE.

<sup>b</sup>Using the average birth date as d 0.

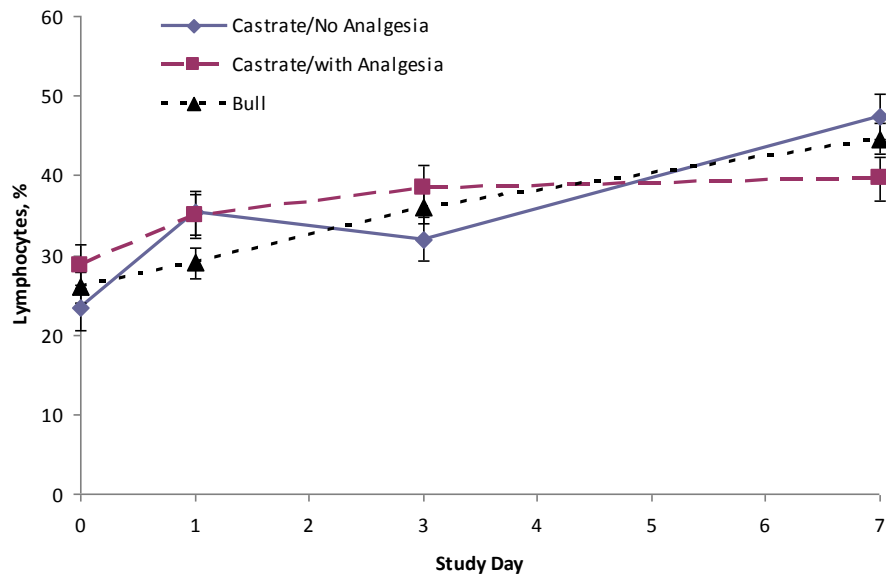
<sup>c</sup>Average daily gain from birth to d 214 (weaning).



**Fig. 1. Effect of castration, with or without analgesia, on white blood cell count. Effect of treatment ( $P = 0.47$ ), day ( $P = 0.002$ ), treatment  $\times$  day ( $P = 0.13$ ).**



**Fig. 2. Effect of castration, with or without analgesia, on percentage of neutrophils. Effect of treatment ( $P = 0.76$ ), day ( $P < 0.0001$ ), treatment  $\times$  day ( $P = 0.01$ ).**



**Fig. 3. Effect of castration, with or without analgesia, on the percentage of lymphocytes. Effect of treatment ( $P = 0.79$ ), day ( $P < 0.0001$ ), treatment  $\times$  day ( $P = 0.02$ ).**

# Intake, digestibility and in situ disappearance of bermudagrass hay diets supplemented with different types of distillers' grains for lactating cows

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## Story in Brief

Distillers' dried grains with solubles may vary substantially in their nutrient composition and availability, primarily because of different processing methods used. This variation may impact the value of distillers grains as supplements for ruminants offered low and medium-quality hay diets. Our objective was to determine the impacts of supplementation with distillers' grains from different sources on forage intake and digestibility of a bermudagrass hay basal diet by lactating beef cows. Four, 8-year-old, multiparous, ruminally cannulated, lactating beef cows (1175 ± 14.0 lb average body weight) were offered 1 of 4 diets during each of 4 periods in an experiment with a 4 × 4 Latin Square design. Cows were housed and fed in individual 400-ft<sup>2</sup> pens with wood chip bedding and were given ad libitum access to bermudagrass hay with either no supplement or 0.45% of body weight (dry matter basis) from either conventional, a low-fat, or a heated low-fat distillers' dried grains plus solubles. Nylon bags containing ground bermudagrass hay were inserted in reverse order into the rumen beginning on d 11 and continued through d 16 for incubation times of 132, 84, 60, 48, 36, 28, 20, 12, 6, and 0 h each period. Hay dry matter intake was greater ( $P < 0.05$ ) by cows offered the hay-only diet compared with those offered conventional or the lower-fat distillers grains, but total dry matter intake and digestibility of dry matter, neutral-detergent fiber, and crude protein did not differ ( $P \geq 0.30$ ) among treatments. Therefore, when offered at these levels, variation in types of distillers grains does not seem to have a substantial effect on intake and digestibility by lactating beef cows offered medium quality bermudagrass hay.

## Introduction

A number of companies process grains to make ethanol from the starch in those grains. By removing the starch, most of the other nutrients are concentrated, resulting in a residue that is high in protein, fat, and phosphorus. This residue is often dried, resulting in dried distillers' grains plus solubles (DDGS). The methodology of this entire process varies among different processing plants including excessive heating on occasion as well as removal of much of the fat from the DDGS. Considerable research has been conducted on DDGS resulting from the consumable alcohol industry, but that fermentation process is somewhat different from that of producing ethanol for fuel. Typical bermudagrass hay may be deficient in energy and sometimes protein for lactating beef cows. Co-product feed-stuffs such as DDGS offer a lower-cost alternative for supplemental energy for lactating cows than feeding cereal grains to meet energy deficiencies for lactating beef cows. The objective of this research was to determine the effects on intake and digestibility of supplementation with different types of DDGS to lactating beef cows offered a bermudagrass hay diet.

## Materials and Methods

Four, 8-year-old, multiparous, ruminally cannulated, lactating beef cows (1175 ± 14.0 lb average body weight (BW)) were housed and fed in individual 400-ft<sup>2</sup> pens with wood chip bedding and were given ad libitum access to bermudagrass hay (Table 1) from large

round bales. Cows were housed in an enclosed facility that allowed air circulation. The cows calved in mid-October and the experiment began March 1 of the following year. The experiment consisted of 4, 16-day periods. Each day, the calves were allowed to nurse the cows at 7:45 AM and 4:30 PM, then were removed and placed on a mixed-grass pasture. This was done to prevent the calves from consuming the diets offered to the cows. Cows and calves were co-mingled and offered bermudagrass hay in a large drylot pen for 5 days at the end of each 16-day period. Manure was removed from the pens twice daily and fresh bedding material was added as needed.

Over the course of the entire study, each cow was offered each of 4 diets during 1 of the 4 periods in an experiment with a 4 × 4 Latin Square design. Diets consisted of bermudagrass hay without supplement (HAY) or supplementation with 0.45% of BW (dry matter (DM) basis) from either conventional DDGS (CDDG), a low-fat DDGS (LFDDG), or a heated LFDDG (HDDG). The HDDG was created under controlled conditions by adding 20% water to LFDDG and heating it in covered aluminum pans for 3 hours at 300 °F.

Cows were offered their respective supplement at 8 AM and allowed approximately 30 minutes to consume the supplement. Bermudagrass hay was then offered throughout the day to maintain ad libitum consumption (minimum of 10% refusal) and unconsumed bermudagrass hay was removed daily at 8 AM. Water was supplied ad libitum and approximately 4 oz. of a commercial mineral supplement<sup>3</sup> (Purina Wind and Rain All Season 4, Purina Mills, Gray Summit, Mo.) was offered to each cow including the CONT cow at 8 AM daily.

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<sup>3</sup> 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 min 13 ppm; Vitamin A min 176,000 IU/lb.; Vitamin D min 44,000 IU/lb.; Vitamin E min 220 IU/lb.

Cows were offered their respective diets for a 10-day adaptation period followed by a 5-day period of collection of fecal grab samples at 8 AM and 4:30 PM. Samples of hay, supplement, rejected feed, and fecal grab samples were taken daily and dried (122 °F) to determine DM. Feed, fecal, and rejected hay samples were analyzed for acid-detergent insoluble ash as an internal digestibility marker to determine DM digestibility. Samples were analyzed for neutral detergent fiber (NDF) and crude protein (CP) and their respective digestibilities were calculated as well.

Nylon bags containing ground bermudagrass hay were inserted in reverse order into the rumen beginning on d 11 and continued through d 16 for incubation times of 132, 84, 60, 48, 36, 28, 20, 12, 6, and 0 h each period. The DM remaining in each bag was used to estimate the effects of the different diets on rate and extent of ruminal DM disappearance.

*Statistical Analysis.* Intake and digestibility data were analyzed using mixed-models procedures of SAS (SAS Institute, Inc., Cary, N.C.) for a 4 × 4 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 (PDIF option) at the respective  $P$ -value.

The proportion of DM remaining in the in situ bags at each incubation time was fit to a non-linear statistical model using PROC NLIN of SAS (SAS Institute, Inc.). The fraction that was degraded at a measurable rate, the digestion lag time, the rate of DM digestion, and the undegradable fraction were derived directly from the model whereas the immediately-soluble (water soluble) fraction was calculated as 100 minus the potentially degradable fraction minus the undegradable fraction. Data derived from the non-linear model were analyzed using mixed-models procedures of SAS (SAS Institute, Inc.) as described previously.

## Results and Discussion

The bermudagrass hay used in this study had high concentrations of NDF but also of CP (Table 1). The CDDG used in this study was similar in CP and NDF concentrations to values reported in NRC (2000). Crude protein and NDF concentrations were both lower from LFDDG and HDDG compared with CDDG. Heat damage as measured by acid-detergent insoluble crude protein (ADICP) was

similar between LFDDG and CDDG, but was 31% greater from HDDG compared with the original LFDDG.

Hay DMI (% of BW) was greater ( $P < 0.05$ ) by cows offered HAY compared with those offered LFDDG and CDDG (Table 2). Hay DMI by cows offered HDDG was not different ( $P > 0.10$ ) from that by cows offered the other diets. Total DMI was not different ( $P = 0.61$ ) across any of the treatments, indicating that supplement intake resulted in a similar reduction in hay intake. Intake of NDF followed a similar pattern to that observed with DM; hay NDF intake by cows offered HAY was greater ( $P < 0.05$ ) than that by cows offered LFDDG and CDDG, but total NDF intake did not differ ( $P = 0.27$ ) across diets.

Digestibility of DM, NDF, and CP did not differ ( $P \geq 0.30$ ) across diets. Since total intake and digestibility did not differ, intake of digestible DM did not differ ( $P = 0.25$ ) across diets.

In situ DM digestibility measurements of a common forage are a good metric for assessing the rumen environment for limitations or benefits of supplementation practices. No dietary differences were observed ( $P \geq 0.35$ ) for the potentially-degradable DM fraction, the digestion lag time, or the rate of DM digestion, indicating that the different supplements were not either enhancing or suppressing microbial activity and overall ruminal digestibility (Table 3).

## Implications

Supplementation with different types of distillers' grains plus solubles to lactating beef cows offered bermudagrass hay that was high in fiber but also high in protein did not improve overall intake. In fact, hay intake was reduced in proportion to the amount of supplement offered, regardless of the type of supplement. However, since the energy derived from the supplements should be greater than the energy derived from the hay, overall energy balance should be improved through supplementation with the distillers' grains plus solubles supplements

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**Table 1. Chemical composition of bermudagrass hay and supplements offered to lactating, ruminally cannulated cows (DM basis)<sup>1</sup>.**

Item	Bermudagrass			
	Hay	LFDDG	HDDG	CDDG
CP	17.7	27.2	26.9	32.8
NDF	73.3	40.5	39.7	46.2
ADICP, % of DM <sup>‡</sup>	NA	9.4	12.3	7.7
ADICP, % of total CP	NA	31.2	41.3	29.7

<sup>1</sup> LFDDG = low-fat distillers' grains plus solubles; HDDG = heated, low-fat distillers' grains plus solubles; CDDG = conventional distillers' grains plus solubles; NA = not analyzed.

<sup>‡</sup> ADICP = acid-detergent insoluble crude protein and is used as an indicator of heat-damaged protein.

**Table 2. Intake and digestibility by lactating beef cows offered bermudagrass hay ad libitum and supplemented with different types of distillers' dried grains plus solubles.**

Item <sup>‡</sup>	Diets <sup>†</sup>				SE <sup>§</sup>
	HAY	LFDDG	HDDG	CDDG	
Hay DM intake, lb	33.9 <sup>a</sup>	27.6 <sup>b</sup>	30.4 <sup>ab</sup>	28.6 <sup>b</sup>	2.50
Hay DMI, % BW	2.86 <sup>c</sup>	2.36 <sup>d</sup>	2.58 <sup>cd</sup>	2.47 <sup>d</sup>	0.171
Supplement DMI, % BW	0.0 <sup>d</sup>	0.39 <sup>c</sup>	0.42 <sup>c</sup>	0.43 <sup>c</sup>	0.017
Total DM intake, lb	33.9	32.2	35.2	33.6	2.68
Total DMI, % BW	2.86	2.75	2.99	2.90	0.176
DM digestion, %	63.7	64.8	66.9	61.3	4.92
Digestible DMI, lb	21.2	20.6	23.5	20.4	1.41
Digestible DMI, % BW	1.8	1.8	2.0	1.8	0.15
Hay NDF intake, lb	24.8 <sup>a</sup>	20.2 <sup>b</sup>	22.2 <sup>ab</sup>	20.9 <sup>b</sup>	1.71
Hay NDF intake, %BW	2.1 <sup>c</sup>	1.7 <sup>d</sup>	1.9 <sup>cd</sup>	1.8 <sup>d</sup>	0.12
Total NDF intake, % BW	2.2	1.9	2.1	2.0	0.12
NDF digestibility, %	70.3	69.9	72.1	67.7	4.25
CP digestibility, %	61.9	63.9	65.4	62.8	5.79

<sup>†</sup> HAY = hay only; LFDDG = low-fat distillers' grains plus solubles; HDDG = heated, low-fat distillers' grains plus solubles; CDDG = conventional distillers' grains plus solubles.

<sup>‡</sup> DM = dry matter; DMI = dry matter intake; NDF = neutral-detergent fiber; CP = crude protein.

<sup>§</sup> Standard error of the mean.

<sup>a,b</sup> Means within a row without a common superscript letter differ ( $P < 0.10$ ).

<sup>c,d</sup> Means within a row without a common superscript letter differ ( $P < 0.05$ ).

**Table 3. In situ dry matter disappearance in lactating beef cows offered bermudagrass hay diets that were supplemented with different distillers' dried grains with solubles.**

Item	Diets <sup>†</sup>				SE <sup>§</sup>
	HAY	LFDDG	HDDG	CDDG	
Potentially degradable fraction, %	61.4	60.0	61.3	59.1	2.52
Rate of digestion, /hour	0.0382	0.0484	0.035	0.039	0.00596
Digestion lag time, hours	6.5	7.1	6.0	8.3	2.22
Undegradable fraction, %	21.6	22.8	21.8	23.1	2.37
Water-soluble fraction, %	17.0	17.2	16.9	17.9	0.68

<sup>†</sup> No significant differences were detected ( $P > 0.10$ ).

<sup>‡</sup> HAY = hay only; LFDDG = low-fat distillers' grains plus solubles; HDDG = heated, low-fat distillers' grains plus solubles; CDDG = conventional distillers' grains plus solubles.

<sup>§</sup> Standard error of the mean.



# Isoflupredone acetate as ancillary therapy for bovine respiratory disease in high-risk stocker calves

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## Story in Brief

The objective of this study was to evaluate the use of isoflupredone acetate as ancillary therapy in the treatment of bovine respiratory disease. Crossbred beef steers (n = 103) were acquired from regional auction markets and were transported to the University of Arkansas Stocker and Receiving Unit located near Savoy. Calves were administered a 5-way modified-live virus vaccine, an 8-way clostridial vaccine, and a dewormer at receiving. Calves were observed daily for signs of respiratory illness, and antibiotic treatment was administered if calves displayed signs of illness and rectal temperature was  $\geq 104$  °F. Calves (n = 31) requiring antibiotic treatment for respiratory illness were assigned to either treatment 1 (injection of antibiotic therapy) or treatment 2 (injection of antibiotic therapy with isoflupredone acetate). Both treatment groups were rechecked 48 hours post treatment to determine treatment efficacy. Blood was collected twice (treatment and recheck) via jugular venipuncture to evaluate complete blood count. Weights were recorded over the 46-day trial to assess weight gain. No difference in average daily gain ( $P = 0.52$ ) or rectal temperature ( $P \geq 0.73$ ) was evident among treatments. Calves that did not receive isoflupredone acetate had a numerically greater ( $P = 0.63$ ) medical cost (\$2.04) because their repull rate tended to be greater ( $P = 0.10$ ). No difference existed in overall white blood cell count or lymphocytes at treatment ( $P = 0.91$  and  $0.72$ , respectively) or recheck ( $P = 0.73$  and  $0.23$ , respectively). Upon recheck, neutrophils decreased to normal for calves that received only antibiotic therapy but remained above normal for calves that also received isoflupredone acetate. At recheck, the neutrophil to lymphocyte ratio was greater ( $P = 0.03$ ) in calves that received isoflupredone acetate. Monocytes at recheck were greater ( $P = 0.02$ ) in calves that did not receive isoflupredone acetate. Results indicate that fewer calves required subsequent antibiotic treatment when receiving an injection of isoflupredone acetate in addition to antibiotic therapy.

## Introduction

Bovine respiratory disease (BRD) is the leading cause of illness and death in U.S. cattle. The disease results from a complex interaction between infectious viral and bacterial pathogens, the environment, and the host. It is often initiated when an animal is exposed to one or multiple stress contributors which cause the animal's immune system to be suppressed, allowing viral or bacterial agents to initiate infection in the body.

Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to be a useful method for treating BRD when used in adjunct to antibiotics (Lockwood et al., 2003; Friton et al., 2005). These drugs do not impair the immune system, and they have pain and fever reducing effects.

Corticosteroid pharmaceuticals have also been used as ancillary therapy, but studies have yielded conflicting results (Bednarek et al., 2003; Sustronck et al., 1997). These drugs have anti-inflammatory properties, and isoflupredone acetate is a corticosteroid that has shown notable results when used as ancillary therapy in treatment of BRD in a challenge model study conducted by Hewson et al., 2011. Isoflupredone acetate is approved in the U.S. for food animal use and has label indications for critical infections in cattle. Therefore, the study objective was to evaluate the use of isoflupredone acetate as ancillary therapy in the treatment of naturally occurring bovine respiratory disease in newly received stocker calves.

## Materials and Methods

Crossbred beef steers (n = 103) were acquired from regional auction markets and were transported to the University of Arkansas Stocker and Receiving Unit located near Savoy. Upon arrival (day

-1), calves were weighed, received an ear tag, and were tested for persistent infection with bovine viral diarrhea virus (PI-BVDV) using the ACE ear notch test (CattleStats -LLC, Oklahoma City, Okla.). Calves were then stratified by body weight and allocated randomly to 1 of 8 pens such that average pen weights were similar. On day 0, calves were weighed again and they received a 5-way modified-live virus vaccine (Pyramid 5<sup>®</sup>, Boehringer Ingelheim Vetmedica, St. Joseph, Mo.), an 8-way clostridial vaccine (Covexin 8<sup>®</sup>, Merck Animal Health, Summit, N.J.), and a dewormer (Cydectin<sup>®</sup>, Boehringer Ingelheim Vetmedica, St. Joseph, Mo.). During the 46-day trial, calves were monitored daily (~8:00 a.m.) for signs of BRD. If 2 or more signs of clinical illness existed (i.e. depression, decreased appetite, coughing, nasal discharge), calves were pulled from the group and rectal temperature was recorded via digital thermometer (GLA Agricultural Products, San Luis Obispo, Calif.). If rectal temperature was  $\geq 104$  °F, calves were treated according to a predetermined antimicrobial protocol consisting of either treatment 1: [florfenicol (n = 17; Nuflor<sup>®</sup>, Merck Animal Health, Summit, N.J.)] or treatment 2: [florfenicol plus isoflupredone acetate (n = 14; Predef 2X<sup>®</sup>, Pfizer Animal Health)].

Both treatment groups were rechecked 48 hours post treatment to determine treatment efficacy. If clinical signs persisted and rectal temperature was  $\geq 104$  °F, then follow-up antibiotic therapy was administered. Calves received enrofloxacin (Baytril<sup>®</sup>, Bayer Animal Health, Shawnee Mission, Kan.) as a secondary treatment and ceftiofur hydrochloride (Excenel<sup>®</sup>, Pfizer Animal Health, Kalamazoo, Mich.) as a tertiary treatment. Vaccine and antimicrobial handling and administration followed Beef Quality Assurance guidelines and manufacturer dosage recommendations.

In addition to weight being recorded upon treatment and recheck, it was also recorded on days 14, 28, 45, and 46. Blood

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was collected (7 mL) twice (treatment and recheck) via jugular venipuncture into evacuated tubes (Vacutainer®, BD Inc, Franklin Lakes, N.J.) containing EDTA to evaluate complete blood count. Over the 46-day trial, calves were fed an identical feed ration of up to 4 lb per day per calf and were given ad libitum access to bermudagrass hay and water. The predetermined quantity of feed was hand fed each morning (~8:30 am).

Data were analyzed using the Mixed Models procedure (PROC MIXED) of SAS (SAS Inst. Inc., Cary, N.C.). Statistical significance was considered for a *P*-value of less than or equal to 0.05.

## Results and Discussion

During processing, no calves tested positive for PI-BVDV. No difference in average daily gain (*P* = 0.52) or rectal temperature (*P* ≥ 0.73) was evident among treatment groups during the 46-day trial. Though not significant, the difference in medical cost between treatment groups was \$2.04 per head (*P* = 0.63). Calves that did not receive isoflupredone acetate had a numerically greater medical cost than calves that received isoflupredone acetate in addition to the antibiotic because of a greater tendency (*P* = 0.10) for those calves to be treated with a second or third antibiotic. The repull rate for calves that did not receive isoflupredone acetate was 14% (7 out of the 17 animals), whereas the repull rate for calves that received isoflupredone acetate was 7% (1 out of the 14 animals).

No difference existed in overall white blood cell count or lymphocytes at treatment (*P* = 0.91 and 0.72, respectively) or recheck (*P* = 0.73 and 0.23, respectively). Upon recheck, neutrophils decreased to normal for calves not receiving isoflupredone acetate but remained above normal for those that received isoflupredone acetate (normal = 0.6 to 4.0 × 10<sup>3</sup>/μL). During infection, an increase in neutrophils is to be expected due to their migration into tissues to destroy pathogenic bacteria (Morris, 2009). After receiving treatment, neutrophils should decrease as the bacteria become less prevalent. No difference was evident in lymphocytes between groups at treatment (*P* = 0.72) or recheck (*P* = 0.23). For both treatment groups, the neutrophil to lymphocyte ratio at recheck was above the normal range (0.3-0.6), and it was greater (*P* = 0.03) in calves that received isoflupredone acetate. This was to be expected since the post treatment neutrophil count in calves receiving isoflupredone acetate tended to be greater (*P* = 0.07) than in calves not receiving isoflupredone acetate. A greater neutrophil

to lymphocyte ratio is associated with more stressed cattle. Monocytes at recheck were greater (*P* = 0.02) in calves that did not receive isoflupredone acetate. These cells circulate in the blood for 1 to 3 days then enter tissues and convert into macrophages. The primary functions of tissue macrophages include removing dead and damaged tissue, tissue repair and remodeling, and regulation of the immune response (Morris, 2009).

## Implications

Results indicate that fewer calves required subsequent antibiotic treatment when receiving an injection of isoflupredone acetate. Further investigation of isoflupredone acetate is needed to evaluate effects on treatment cost and post treatment gains in newly received stocker calves.

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**Table 1. Effects of isoflupredone acetate as ancillary therapy for bovine respiratory disease on morbidity and growth performance.**

	Antibiotic treatment	Antibiotic treatment with isoflupredone acetate	<i>P</i> -value
Repull rate, %	41.2	7.1	0.10
Time to second pull, days	9	7	0.59
Treated twice, calves	4	1	0.17
Treated thrice, calves	3	0	0.12
Medical cost, \$	20.13	18.09	0.63
Temperature at treatment, °F	104.8	104.7	0.73
Temperature at recheck, °F	103.0	103.0	0.99
Change in Temperature, °F	1.8	1.7	0.85
Average daily gain, lb	2.2	2.0	0.52
Gain total over 46-day study, lb	101.0	91.9	0.52

**Table 2. Effects of isoflupredone acetate as ancillary therapy for bovine respiratory disease on blood count analysis.**

	Antibiotic treatment	Antibiotic treatment with isoflupredone acetate	P-value
<b>Initial</b>			
White blood cells, n x 10 <sup>3</sup> /μL	11.0	11.2	0.91
Neutrophils, n x 10 <sup>3</sup> /μL	4.2	4.1	0.92
Lymphocytes, n x 10 <sup>3</sup> /μL	3.8	4.0	0.72
Neutrophil:Lymphocyte, %	121	118	0.94
Monocytes, n x 10 <sup>3</sup> /μL	0.9	0.8	0.30
Platelets, n x 10 <sup>3</sup> /μL	376.8	306.6	0.03
<b>Recheck</b>			
White blood cells, n x 10 <sup>3</sup> /μL	10.0	10.4	0.73
Neutrophils, n x 10 <sup>3</sup> /μL	3.2	4.2	0.07
Lymphocytes, n x 10 <sup>3</sup> /μL	4.1	3.5	0.23
Neutrophil:Lymphocyte, %	86	129	0.03
Monocytes, n x 10 <sup>3</sup> /μL	1.0	0.8	0.02
Platelets, n x 10 <sup>3</sup> /μL	381.1	367.8	0.73

# Estrous response and pregnancy rates in beef cows following a 6 or 7 day controlled internal drug release synchronization protocol

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## Story in Brief

Estrous synchronization protocols, where progesterone treatment is removed 24 hours after prostaglandin administration, may delay estrus in some cows and result in a more synchronized estrus than where progesterone treatment is removed at time of prostaglandin administration. This study compared estrus response and pregnancy rates of lactating beef cows synchronized with a 6 or 7 day controlled internal drug release (CIDR) progesterone insert and bred via artificial insemination. Sixty-one cows were assigned to treatment based on parity, body weight, body condition score, cyclicity, and postpartum interval. All cows received a CIDR on day 0 and prostaglandin on day 6. The CIDR inserts were removed on day 6 or 7 in treatment 1 and 2 cows, respectively. Cows were observed for estrus and inseminated 8 to 24 hours following onset of estrus. Cows not responding to treatments were treated with gonadorelin and fixed-time inseminated at 96 hours. One week following the end of estrus detection, all cows were placed with fertile bulls for 45 days. Pregnancy rates were determined via ultrasonography approximately 45 d following insemination and 30 d following removal of bulls. Removal of CIDR inserts 1 day after prostaglandin (PGF) (Treatment 1) delayed ( $P < 0.01$ ; 69.0 versus 52.3 hours, respectively) the onset of estrus after PGF but resulted in better estrus synchrony. In Treatment 2, 100% of cows that exhibited estrus did so within a 12-hour period versus 75% in treatment 1. Across treatments, the number of cows detected in estrus following synchronization, as well as artificial insemination and season pregnancy rates, were similar ( $P = 0.61, 0.46$  and  $0.53$ , respectively). Data indicates removal of CIDR progesterone inserts 24 hours after prostaglandin administration may result in tighter synchronization of cows over a 12-hour period, without adversely affecting estrus expression or pregnancy rates.

## Introduction

Estrous synchronization is a reproductive management tool used to control the estrous cycle, allowing a group of females to express estrus and ovulate within a short amount of time (Seidel, 1995). Together with artificial insemination (AI), these biotechnologies have been reported to be the most important technological advancements affecting the beef and dairy cattle industry (Seidel, 1995). Although these technologies have been available to beef producers for over 30 years, few small-scale producers utilize these technologies. In order to make estrous synchronization and AI more appealing to producers, estrous synchronization protocols must be more cost effective and easier to use, by allowing for fixed-time AI. An estrous synchronization protocol utilizing removal of controlled internal drug release (CIDR) progesterone inserts 24 hours after prostaglandin (PGF) administration should delay estrus in some cows and result in a more synchronous estrus than protocols where CIDRs are removed at time of PGF injection. Therefore, the objective of this study was to compare estrous response and subsequent pregnancy rates of lactating beef cows where CIDR removal occurred at or 1 day after PGF treatment.

## Materials and Methods

Multiparous Angus and Angus  $\times$  Hereford crossbred cows ( $n = 61$ ) were randomly assigned to treatments based on parity, body weight, body condition score, cyclicity, and the number of days postpartum. All cows received an Eazi-Breed CIDR progesterone insert on day 0 and were administered 25 mg Lutalyse (PGF) on day 6. The CIDR inserts were removed from treatment 1 cows ( $n = 30$ ) on d 6 (at the time of PGF administration) or 24 hours following PGF administration in Treatment 2 cows ( $n = 31$ ). All cows received

an Estroject estrus detection patch at the time of CIDR removal and were monitored for estrus behavior over a 72-hour period. Cows detected in estrus were inseminated 8 to 24 hours following onset of estrus. Cows not detected in estrus during the 72-hour period were administered 100  $\mu$ g of gonadorelin (GnRH, Factrel) at 96 hours and inseminated (fixed-time cleanup AI). One week after the last inseminations, all cows were exposed to fertile bulls for 45 days. Artificial insemination pregnancy rates were determined via transrectal ultrasonography 45 d following the AI period and season pregnancy rates were determined 30 d following removal of bulls. Statistical analysis was performed using analysis of variance and Chi-square. Analysis of variance was used to determine any differences in the interval from CIDR removal to estrus between treatments while Chi-square was used to determine effects of treatment upon estrus response, AI pregnancy, and seasonal pregnancy rates.

## Results and Discussion

When the estrous cycles of cows are synchronized, the majority of cows usually express estrus 48 to 72 hours after PGF treatment, but some cows will express estrus as early as 24 hours after treatment. Delaying the removal of CIDR progesterone inserts until 24 hours after PGF treatment should also delay estrus in those cows that might express estrus early, resulting in better estrus synchrony. The mean interval from PGF treatment to estrus was greater ( $P < 0.01$ ; 69.0 versus 52.3 hours, respectively) when CIDR removal occurred 24 hours after PGF (Treatment 2) versus CIDR removal concurrent with PGF treatment (Treatment 1; Table 1). This delay in estrus resulted in 100% of cows that exhibited estrus, doing so within a 12-hour period in Treatment 2 versus 74% in Treatment 1. The AI pregnancy rate for cows detected in estrus and inseminated

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was similar ( $P = 0.46$ ) for cows in Treatments 1 and 2 (65.2 and 54.5%, respectively). The cleanup (timed) AI of cows not detected in estrus but given an injection of GnRH and inseminated at 96 hour after PGF treatment resulted in a pregnancy rate of 50% (8/16) and was similar ( $P = 0.61$ ) across treatments. Overall, a single insemination resulted in 35 of 61 (57.4%) of cows. At the end of the breeding season 93.3 and 96.8% of the cows in Treatments 1 and 2, respectively were pregnant ( $P = 0.53$ ). The results of this study indicate removal of CIDR progesterone inserts 24 hours after prostaglandin administration may result in tighter synchronization of cows over a 12-hour period, without adversely affecting estrus expression or pregnancy rates. The tighter synchrony would benefit AI programs where all cows are inseminated at a fixed with without estrus detection.

## Implications

Delaying removal of CIDR progesterone inserts until 24 hours after administration of prostaglandin F2alpha delayed estrus, resulting in tighter estrus synchronization of cows over a 12-hour period. The tighter synchrony would benefit AI programs where all cows are inseminated at a fixed with without estrus detection.

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**Table 1. Effects of 6 or 7 d CIDR upon estrus response, AI pregnancy, and seasonal pregnancy rates.**

Description	Synchronization treatment		P Value
	6 d CIDR	7 d CIDR	
Estrus response (%)	23/30 (76.7%)	22/31 (71.0%)	0.61
Interval, CIDR removal to estrus (h)	52.3 ± 1.6	45.0 ± 1.6	0.01
AI pregnancy rate after detected estrus	15/23 (65.2%)	12/22 (54.6%)	0.46
Seasonal pregnancy rate (%)	28/30 (93.3%)	30/31 (96.8%)	0.53

# Influence of growth-promoting implants on development of low weight replacement beef heifers

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## Story in Brief

Charolais × Balancer heifer calves (n = 66; 393 ± 67 lb; 255 ± 12 days of age) were used to determine the influence of implants on growth and reproductive development of low weight replacement beef heifers. Heifers were blocked by body weight and assigned to 1 of 4 implant treatment groups: 1) control, no implant (C; n = 16); 2) trenbolone acetate (TBA; n = 16); 3) trenbolone acetate plus estradiol (TBA+E2; n = 17); or 4) zeranol (ZER; n = 17). All heifers grazed mixed pastures as a single group for 106 days. Heifers were implanted on day 0, and body weight, hip height and body condition score were recorded on day 0 and 106. Reproductive tract scores (RTS; on a scale of 1 to 5 where scores of 1, 2, and 3 were classified as noncyclic, and scores of 4 and 5 were considered cyclic) of heifers were determined via ultrasonography on day 106. Body weight, body condition and hip height were not influenced ( $P > 0.10$ ) by implant treatment. Body weight change was greater ( $P < 0.03$ ) in the heifers implanted with trenbolone acetate plus estradiol; changes in body condition score and hip height of heifers were not different ( $P > 0.10$ ) among implant treatment groups. Reproductive tract scores were affected by treatment ( $P = 0.04$ ). A lower percentage ( $P < 0.05$ ; 18%) of heifers treated with zeranol were classified with a cyclic reproductive tract score on day 106 than control heifers (53%) and heifers treated with trenbolone acetate (63%); heifers treated with trenbolone acetate plus estradiol (35%) were similar ( $P > 0.10$ ) to all implant treatment groups. Growth-promoting implants may impact body weight gain and reproductive development of low weight replacement heifers.

## Introduction

Proper development of replacement heifers is essential to maintain a profitable cow herd. Replacement heifers should reach puberty at an early age to assure high conception rates in their first breeding season (Lesmeister et al., 1973). Weight is a major factor affecting age at puberty, and as a result, heifers fail to reach puberty until significant weight gains are made (Patterson et al., 1992). Adequate gains and skeletal development are necessary to optimize replacement heifer development.

Various growth-promoting implants used in suckling calves are available to increase gain; however, few are recommended for replacement heifers due to the possible detrimental effects on fertility and subsequent productivity. With the U.S. cattle herd the lowest since 1952, a more aggressive expansion of replacement heifer retention is necessary. The largest proportion of the cost of replacement heifer development is the cost of retaining the calf. Beef producers may want to consider adding value to low weight heifers to retain replacements. Our objective was to determine the influence of growth-promoting implants on growth and reproductive development of replacement beef heifers.

## Materials and Methods

The University of Arkansas' Institutional Animal Care and Use Committee (#13021) approved the animal procedures used in this study. Spring-born, Charolais × Balancer heifers calves (n = 66; 393 ± 67 lb; 255 ± 12 days of age) from the UA, Livestock and Forestry Research Station in Batesville, Ark. were transported to the UA, North Farm, Fayetteville, Ark. on 26 October 2012. Heifers were blocked by body weight (BW) and assigned (15 November 2012) to 1 of 4 implant treatment groups: 1) control, no implant (C; n = 16); 2) trenbolone acetate (TBA, Component<sup>®</sup> TH with Tylan<sup>®</sup>

and 200 mg of trenbolone acetate, Ivy Animal Health, Overland Park, Kan.; n = 16); 3) trenbolone acetate plus estradiol (TBA+E2, Component<sup>®</sup> TE-G with Tylan<sup>®</sup> and 40 mg trenbolone acetate and 8 mg estradiol, Ivy Animal Health, Overland Park, Kan.; n = 17); or 4) zeranol (ZER, Ralgro<sup>®</sup> with 36 mg zeranol, Intervet Inc., Merck Animal Health, Summit, N.J.; n = 17). Ear notches were collected from each heifer and submitted to a commercial laboratory (Cattle Stats, LLC, Oklahoma City, Okla.) for determination of bovine viral diarrhea virus persistent infection. All heifers rotationally grazed orchard grass, novel endophyte-infected tall fescue, and mixed grass pastures as a single group for 106 days and were supplemented with alfalfa haylage (daily average = 1.1 lb/heifer on an as-fed basis) from 7 January to 25 February when available forage was limited.

Heifers were implanted once according to treatment group on day 0. Growth measurement data including BW, hip height (HH) and body condition score (BCS; 1 = very thin to 9 = obese) were determined at day 0 (15 November) and 106 (1 March) of grazing. Hip height was measured using a sliding caliper which was developed specifically to measure external body dimensions in beef cattle (Altitude Stick, NASCO, Fort Atkinson, Wis.).

Reproductive tract scores (RTS; scale of 1 to 5; Anderson et al., 1991) of heifers were determined via ultrasonography (Aloka 500 V<sup>®</sup>; Corometrics, Wallingford, Conn., equipped with a 5.0-MHz transducer) on day 106. A RTS of 1 describes an immature reproductive tract with no uterine tone and no palpable ovarian structures. An RTS 2 is a heifer with ovaries exhibiting 8 mm follicles and a uterine horn diameter of 20 to 25 mm with no uterine tone. Heifers scored a RTS 3 have an ovarian follicle from 8 to 10 mm with uterine tone and a uterine horn diameter of 25 to 30 mm. An RTS 4 describes uterine horns 30 mm in diameter with good tone, ovarian follicles that are >10 mm, and possibly a corpus luteum present. A RTS of 5 describes a cycling heifer with a functional corpus luteum. In the current experiment, scores of 1,

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2, and 3 were classified as noncyclic while scores of 4 and 5 were considered cyclic. Heifers with BW < 500 lb were not ultrasounded to avoid possible injury to the heifers and categorized as a RTS 2 (Patterson et al., 1994).

Growth performance parameters were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, N.C.) with heifer as the experimental unit. Influence of implant treatment group on RTS was analyzed by Chi-square using FREQ procedure of SAS. Treatment means were reported as least squares means. Least squares means were compared using the PDIF statement of SAS when protected by a significant ( $P < 0.05$ ) treatment effect.

## Results and Discussion

All heifers were diagnosed as negative for persistent infection with bovine viral diarrhea virus. Growth measurements of heifers are shown in Table 1. Body weight, BCS, and HH were not affected ( $P > 0.01$ ) by implant treatment after 106 days of grazing (Table 1). However, BW change of TBA+E2-implanted heifers was greater ( $P < 0.03$ ; average change = +243 ± 8 lb) compared with all other implant groups (average change = +213 ± 8 lb; Table 1). It is well established that with adequate nutrition, implants increase BW gains usually from 4% to 20%. Average daily gain (ADG) of TBA+E2-implanted heifers was greater ( $P < 0.05$ ; 2.3 ± 0.1 lb/day) than all other implant treatment groups (average ADG = 2.0 ± 0.1 lb; Table 1) following 106 days of grazing. Changes in BCS and HH of heifers were not different ( $P > 0.10$ ) among implant treatment groups following 106 days of grazing.

Reproductive tract scoring is used to evaluate sexual maturity of heifers and is accomplished through rectal palpation of a heifer's reproductive organs. Size and development of the uterus and ovaries will assess the heifer's reproductive maturity (Anderson et al., 1991). Impact of growth-promoting implants on heifer reproductive performance appears to be age and weight dependent. Heifers implanted with zeranone at 1, 6, or 9 months had an increased incidence of non-ovulatory estrus than non-implanted heifers (Deutscher et al., 1986); however, conception rates were similar between implanted and non-implanted heifers. In the current

experiment, RTS was affected by treatment ( $P = 0.04$ ). Less ( $P < 0.05$ ) heifers treated with ZER were classified with a cyclic reproductive tract score (RTS of 4 or 5) on day 106 than C heifers and heifers treated with TBA; heifers treated with TBA+E2 were similar ( $P > 0.10$ ) to all other implant treatment groups (Fig. 1).

## Implications

Low weight replacement beef heifers treated with trenbolone acetate plus estradiol had greater body weight change, and consequently enhanced average daily gain. Heifers implanted with zeranone at the start of grazing were less likely to be classified with a cyclic reproductive tract score after 106 days compared with control or trenbolone acetate-implanted heifers. It would appear that growth-promoting implants that enhance weight gain may be detrimental to reproductive tract development. Strategic use of implants that increase heifer growth without jeopardizing fertility could be economically advantageous to beef producers. Producers always should read and follow implant label directions.

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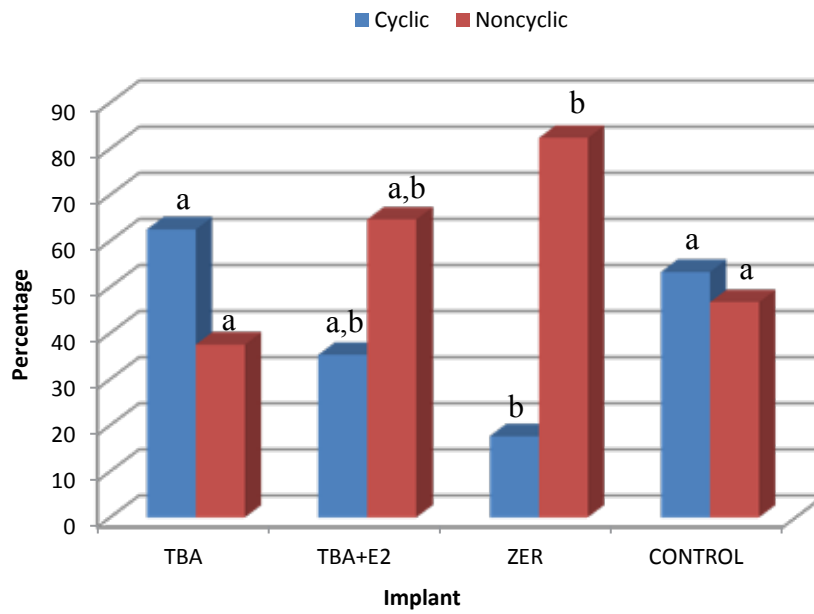
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**Table 1. Body weight (BW), BW change, average daily gain (ADG), body condition score (BCS), BCS change, hip height (HH) and HH change of replacement, crossbred beef heifers not implanted (C), or implanted with trenbolone acetate (TBA), trenbolone acetate plus estradiol (TBA+E2) or zeranone (ZER).**

Item	Treatment				S.E. <sup>†</sup>	P-Value
	Control	TBA	TBA+E2	ZER		
BW, d 0, lb	398	395	383	398	17	0.91
BW, d 106, lb	611	606	626	612	21	0.92
BW change, lb	214 <sup>a</sup>	210 <sup>a</sup>	243 <sup>b</sup>	214 <sup>a</sup>	8	0.03
ADG, lb	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.3 <sup>b</sup>	2.0 <sup>a</sup>	0.1	0.03
BCS, d 0	4.5	4.5	4.4	4.4	0.1	0.90
BCS, d 106	5.0	4.9	5.1	5.1	0.1	0.50
BCS change	0.5	0.4	0.7	0.6	0.1	0.40
HH, d 0, in	43.0	42.8	43.0	43.0	0.5	0.99
HH, d 106, in	45.0	45.1	45.1	45.3	0.5	0.98
HH change, in	2.0	2.2	2.2	2.4	0.2	0.80

<sup>†</sup> Pooled standard error.

<sup>a,b</sup> Unlike superscripts within rows indicate difference ( $P < 0.03$ ).



**Fig. 1.** Influence of trenbolone acetate (TBA; n = 16), trenbolone acetate plus estradiol (TBA+E2; n = 17), or zeranol (ZER; n = 17) implants; or control, no implant (n = 16) on reproductive tract score in low weight beef heifers. <sup>a,b</sup>Unlike superscripts within category (cyclic or noncyclic) indicate difference ( $P < 0.05$ ).



# Response of Angus cows and their suckling calves to an injectable trace mineral supplement

M.S. Gadberry<sup>1</sup> and B. Baldrige<sup>2</sup>

## Story in Brief

The objective of this study was to examine the response of Angus cows and their nursing calves to an injectable form of trace mineral supplementation in a production environment where free choice, trace-mineral supplementation was not routinely practiced. Cows were assigned to 1 of 2 treatments. Treatment 1 received 0.5 mL/100 lb body weight (BW) Multimin 90 (Multimin 90, Multimin USA, Fort Collins, Colo.) prior to fall calving followed by a second injection prior to breeding (ITM), and cows assigned to treatment 2 did not receive a mineral injection (NITM). At approximately 130 d following birth, one-half of the calves from each cow treatment were given Multimin at 1 mL/100 lb BW. Injectable mineral supplementation did not affect cow BW, body condition score (BCS), pregnancy rate, or postpartum interval. In addition, injectable mineral supplementation did not affect calf weight gain or calf hair coat score. In conclusion, neither Angus cows nor their calves showed a response to the mineral injection.

## Introduction

In the absence of a mineral supplementation program, the average mineral composition of improved forages in Arkansas suggests that trace mineral deficiencies may be marginal and likely result in subclinical losses. Gadberry and Simon (2012a and 2012b) observed no statistically significant improvements in reproduction, calf weaning weight, or calf health when injectable mineral was included in the management of a 50 cow herd. That herd historically had not received any form of free-choice mineral and vitamin supplementation but was managed using rotational grazing with high-quality forages (complimentary warm- and cool-season annual and perennial pastures with some legumes). The objective of this study was to examine cow and calf response to an injectable form of trace mineral supplementation in a production environment whereby free choice salt (not fortified with trace minerals) was historically provided.

## Materials and Methods

This project was conducted in cooperation with the Jim Perkins ranch located in Lawrence county, Arkansas, and the Lawrence county Extension office. Cattle at this facility had free-choice access to salt that was not fortified with trace minerals. Cattle on this ranch are managed in multiple groups and predominately grazing warm-season grass pastures. Although not historically practiced on the ranch, all cows were offered poultry litter beginning December 19, 2011 until March 4th, 2012 to stretch hay supplies. As a result, all cows were exposed to a high rate of trace mineral supplementation during breeding.

One hundred thirty-five fall calving Angus cows (last trimester body weight (BW) 1279 ± 106 lbs) were assigned to either receive injectable mineral (ITM) or not receive injectable mineral (NITM) prior to fall calving and again prior to breeding. Cows were managed in 2 separate groups. Individual female was considered the experimental unit. Group 1 consisted of 60 cows (32 ITM and 28 NITM), and group 2 consisted of 75 cows (37 ITM and 38 NITM). 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 August 12, 2011 (prior to fall-calving) at a dose of 0.5 mL/100 lb BW. A placebo injection was not administered to NITM cows. Females administered ITM were given a second injection November 7, 2011, prior to breeding. On each treatment date, cows were weighed and a body condition score (BCS) assigned using the 1 to 9 scoring system. Breeding was initiated in December, 2011 and consisted of artificial insemination for initial service followed by bull exposure one week later. Bulls remained in the cow herds until August. Rectal palpation was performed by a local veterinarian the following summer to determine each cow's pregnancy status.

Within each cow treatment, approximately 1/2 of the calves were injected with Multimin (1mL/100 lb BW s.c.) on March 3, 2012. A total of 93 calves were available for this component of the study. This resulted in 4 treatments for the calves (calf's treatment:dam's treatment) 1) NITM:NITM (n = 25), 2) NITM:ITM (n = 22), 3) ITM:NITM (n = 22), and 4) ITM:ITM (n = 24). Calves assigned to the NITM treatment did not receive a placebo injection. Calves were weighed at the time of injection and again May 22, 2012. In addition to recording BW, weight per day of age (WDA) was calculated from the initial weight and hair coat scores were assessed when calves were weighed in May. Hair coat scores were based on a 1 to 5 scale with 1 = slick, short summer type coat, 3 = 50% winter coat shed and 5 = 100% winter coat.

Treatment effects for cow BW and BCS were modeled using a generalized linear model. Group was initially included as a fixed effect in the model. Treatment interaction with group was never a significant source of variation; therefore, the final model only included the independent mineral treatment effect. Pearson's chi-square test was used to examine the effect of treatment on the percentage of cows determined pregnant by the rectal palpation method. Postpartum interval was calculated for all cows that had calved when previous calving date was known. Calf gender did not differ among treatments ( $X^2$ ,  $P = 0.53$ ) and was excluded from calf data analysis models. Calf hair coat scores were compared based on a chi-square distribution for count data. Calf age, weight, WDA, and

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BW gain was analyzed using a generalized linear model. Analysis was formed with R software ([www.r-project.org](http://www.r-project.org)).

## Results and Discussion

Sample size differed among response variables for various reasons including, missing cows during gathering at the second weigh date, missing cows during gathering for palpation, or no previous calving date. Cow BW and BCS was similar among treatments at the beginning of the study in August and did not differ between treatments prior to breeding in November ( $P = 0.69$  and  $0.89$  for BW and BCS in November, respectively, Table 1). Average pre-calving BCSs (5.7 and 5.8 for ITM and NITM, respectively) met beef cattle industry targets for body condition at calving. The percentage of cows classified as pregnant by rectal palpation was 89% for the NITM treatment and 95% for the ITM treatment but did not differ statistically ( $X^2$ ,  $P = 0.40$ ). In addition, 83 cows had current and previous calving date records, and the postpartum interval between treatments for these cows only differed by 4 days ( $P = 0.63$ ). The average calving interval for either group averaged 30 days longer than desired. Contrary to expectations, cows were heavier in November compared to their pre-calving BW but BCS was 0.3 to 0.4 units lower. This discrepancy may partially be explained by gut fill at weighing as these cattle were not purposely shrunk or weighed on two sequential dates as sometimes implemented under research facility protocols.

The calf analysis was conducted as a one-factor model that incorporated both the calf's and dam's treatment, instead of a two-way factorial model. For simplicity, the single factor model demonstrates the benefits of no injection, injecting dam only, injecting calf only, or injecting dam and calf. The average age of calves within treatment was statistically similar ( $P = 0.37$ ) when calf treat-

ments were applied in March (Table 2). Weight per day of age did not differ among any treatments ( $P = 0.40$ ), indicating that cow treatments were not affecting calf weight by 4 months of age. In addition, the similarity in WDA demonstrated there was no bias in allocation for the age and weight relationship of calves among calf treatments. Calf BW gain and May BW were similar among treatments,  $P = 0.52$  and  $0.15$ , respectively. Hair coat scores were also assessed in May. The majority of calves were carrying 75% or more of their winter coat (hair coat score 4 and 5) in late May ( $X^2$ ,  $P = 0.35$ ).

## Implications

These results indicate that injectable mineral did not result in an improvement in BW, BCS or pregnancy rate for cows when offered free choice access to salt year and supplemented with poultry litter throughout winter which coincided with the breeding season. In addition, injectable mineral previously given to cows and/or their calves at 130 days of age did not improve calf weight gain or hair coat scores.

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**Table 1. Performance of cows receiving a trace mineral injection prior to fall calving followed by a pre-breeding injection.**

	n	Treatment <sup>a</sup>		Pooled SEM	P-value
		ITM	NITM		
BCS <sup>b</sup>					
August, lb	135	5.7	5.8	0.08	0.18
November, lb	129	5.4	5.4	0.12	0.89
BW					
August, lb	135	1279	1276	18	0.82
November, lb	129	1337	1329	20	0.69
Pregnancy <sup>c</sup> , % positive	113	95	89	---	0.40
Postpartum interval, d	83	392	396	6.7	0.63

<sup>a</sup> ITM (trace mineral injection) and NITM (no trace mineral injection: Multimin 90 injection at 0.5mL/100 lb BW).

<sup>b</sup> BCS (body condition score scale from 1 to 9 whereby 1 = emaciated and 9 = obese).

<sup>c</sup> Pregnancy determined by rectal palpation in late summer and analyzed using a Chi-square test.

**Table 2. Performance of calves receiving a trace mineral injection at 130 d of age.**

	Treatment (calf treatment:dam treatment) <sup>a</sup>				Pooled SEM	P-value
	NITM: NITM	NITM: ITM	ITM: NITM	ITM: ITM		
March age, d	139	130	128	123	6.5	0.37
March WDA <sup>b</sup> , lb BW	1.9	2.1	2.1	1.9	0.08	0.40
March, lb	258	250	267	231	12.4	0.20
May, lb	402	392	414	367	14.8	0.15
Gain, lb	143	142	146	136	5.3	0.52
Hair coat score <sup>c</sup> , May						
Score 5, %	100	86	86	83	---	---
Score 4, %	0	9	9	17	---	---
Score 3, %	0	5	5	0	---	---

<sup>a</sup> ITM (trace mineral injection) and NITM (no trace mineral injection); calves received a single Multimin 90 injection at 1 mL/100 lb BW and dam's previously received 0.5 mL/100 lb BW pre-calving and again pre-breeding.

<sup>b</sup> Weight per day of age.

<sup>c</sup> Hair coat ( $\chi^2$ ,  $P = 0.35$ ) was scored on a 1 to 5 scale with 1 = slick summer coat and 5 = 100% winter coat.

# Effects of copper oxide bolus administration on productivity and copper status in grazing beef calves supplemented with dried distillers' grains

J. Hawley<sup>1</sup>, E.B. Kegley<sup>1</sup>, J.M. Bauer<sup>1</sup>, and J.G. Powell<sup>1</sup>

## Story in Brief

A study was conducted to assess the effects of copper oxide bolus administration on productivity and copper status in grazing beef calves supplemented with dried distillers' grains. Calves (n = 74, yr 1; n = 54, yr 2) were assigned randomly to one of two treatments: 1) a single intraruminal copper oxide bolus (12.5 g) or 2) non-bolus control for a growth trial (141-d, yr 1; 92-d, yr 2). Calves grazed predominantly bermudagrass (*Cynodon dactylon* [L]) pastures. Moreover, dried distillers' grains were offered at a rate of 0.75% of body weight (as fed). Calves were weighed at 28-d intervals and dried distillers' grains were adjusted after each weigh day. Blood samples were collected for plasma copper and zinc concentrations. There was no ( $P = 0.68$ , yr 1;  $P = 0.83$ , yr 2) difference in average daily gain between bolused and non-bolused calves. Administration of the copper oxide bolus did not ( $P = 1.00$ , yr 1;  $P = 1.00$ , yr 2) result in greater plasma copper concentrations compared with non-bolused calves. Accordingly, these results suggest that copper oxide bolus administration is ineffective in grazing beef calves that are supplemented dried distillers' grains 0.75% (as fed) of body weight. Dried distillers' grains at 0.75% (as fed) of body weight did not create sufficient copper antagonism.

## Introduction

Feeding byproduct feeds to cattle is not a new concept. Producers consider byproduct feeds as they are inexpensive sources of protein and energy; however, most are characterized by atypical mineral profiles. Processing of grains and oilseeds has the effect of removing some nutrients from the eventual byproduct while concentrating others. Several popular byproduct feeds (e.g., distiller's grains and corn gluten feed) are characterized by high concentrations of sulfur (S). The maximum tolerable concentration of dietary S is estimated to be 0.5% S for roughage diets (NRC, 2005). Distillers' grains can have concentrations of 0.8% S or greater (Buckner et al., 2008). Sulfur is a potent antagonist of dietary copper (Cu), a trace mineral that plays a large role in the health and reproduction of beef cattle. Copper deficiency generally has the most detrimental effect on young, growing animals. Therefore, to manage byproduct feeds appropriately, methods must be tailored to supplement sufficient Cu. Direct supplementation of minerals in water or supplement results in animal-to-animal variation in the amount of mineral consumed; however, oral dosing of trace mineral boluses ensures each animal receives the prescribed dose. The objective of this study was to investigate the effect of Cu oxide (CuO) bolus administration on productivity and Cu status in beef calves supplemented dried distillers' grains (DDG).

## Materials and Methods

**Animals, Treatments, and Diets—Year 1.** Seventy-four heifer (n = 36) and steer (n = 38) calves (8 to 10 mo of age, initial BW = 564 ± 73.0 lb) of predominantly Angus breeding were obtained from the University of Arkansas cow-calf facility in Savoy for a 141-d growth trial. Calves were stratified by body weight (BW) within gender and assigned randomly to one of two treatments: 1) a single intraruminal CuO bolus (12.5 g CuO needles; Copasure; Animax Ltd., Columbus, Ohio), or 2) non-bolus control. Calves grazed

predominantly dormant bermudagrass (*Cynodon dactylon* [L]) in two groups (by gender). Moreover, calves were provided access to bermudagrass hay (Table 1) in quantities sufficient to ensure ad libitum access. Dried distillers' grains were offered at a rate of 0.75% of BW (as fed) and adjusted based on the average for all calves after each weigh date. Dried distillers' grains were produced at a commercial dry milling plant from the fermentation of 100% corn (MGP Ingredients, Inc., Atchison, Kan.). Daily amounts of DDG were weighed and provided at 0800 h. Supplements offered were entirely consumed. Calves had ad libitum access to water and a trace mineral salt (Champions Choice Selenium "90" Trace Mineral Salt; Cargill, Inc., Minneapolis, Minn.<sup>2</sup>). Calves were monitored daily for morbidity.

**Year 2.** Fifty-four heifer (n = 30) and steer (n = 24) calves (10 to 12 mo of age, initial BW = 696 ± 64.1 lb) of predominantly Angus breeding were obtained from the University of Arkansas cow-calf facility in Savoy for a 92-d growth trial. Calves were randomly assigned to one of 6, 2.4-ha pastures of predominantly bermudagrass (Table 1). Calves were stratified by BW within gender and assigned randomly to one of two treatments: 1) a single intraruminal CuO bolus (12.5 g CuO needles; Copasure; Butler Schein™ Animal Health, Dublin, Ohio), or 2) non-bolus control. Steers were implanted with Component® TE-G with Tylan® (trenbolone acetate 40 mg and estradiol 8 mg; Ivy Animal Health, Inc., Overland Park, Kan.). Dried distillers' grains were offered at a rate of 0.75% of BW (as fed) and adjusted based on the average for all calves after each weigh date. Dried distillers' grains were produced at a commercial dry milling plant from the fermentation of 100% corn (MGP Ingredients, Inc., Atchison, Kan.). Daily amounts of DDG were weighed and provided at 0800 h to calves in their respective pastures with 0.5 m or more bunk space per calf. Supplements offered were entirely consumed. Calves had ad libitum access to water and a trace mineral salt (Powell 4% Beef Mineral; Powell Feed and Milling Co. Inc., Green Forest, Ark.<sup>3</sup>). Calves were monitored daily for morbidity.

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<sup>2</sup> Trace mineral salt composition (mg/kg): 50.0 Cobalt, 300.0 Copper, 70.0 Iodine, 2000.0 Manganese, and 90.0 Selenium.

<sup>3</sup> Trace mineral salt composition: 18.00% calcium, 4.00% phosphorous, 0.20% manganese, 0.10% potassium, 100.00 mg/kg copper, 26.0 mg/kg selenium, and 500.0 mg/kg zinc.

**Sample Collection–Year 1.** Calves were weighed at the beginning and end of the study on two consecutive days. Interim BW were collected every 28 d. Grab samples of the DDG fed to each group were collected daily and composited over 28 d periods within group. Grab samples of the hay fed to each group were collected as bales were fed and composited over 28 d periods within group. Blood samples were collected for plasma Cu and zinc (Zn) concentrations on d 0 and 140 from all calves and on d 28, 56, and 84 from 32 calves (8 calves/gender/treatment) via jugular venipuncture into heparinized tubes.

**Year 2.** Calves were weighed at the beginning and end of the study on two consecutive days. Interim BW were collected every 28 d. Grab samples of the DDG fed to each pasture were collected daily and composited over 28 d periods within pasture. Clipped forage samples (simulated grazed) were obtained every 28 d. Forage intake was not quantified. Blood samples were collected for plasma Cu and Zn concentrations on d 0 and 91 from all calves and on d 35 from 24 calves (6 calves/gender/treatment) via jugular venipuncture into heparinized tubes.

**Laboratory Analysis.** Forage and composited feed samples were analyzed for dry matter, total nitrogen by the rapid combustion procedure, acid detergent fiber (ADF) and neutral detergent fiber (NDF) by batch procedures using the ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Fairport, N.Y.), and mineral content by inductively coupled plasma spectroscopy (Model 3560, Applied Research Laboratory, Sunland, Calif.) after wet ashing (Table 1). Composited DDG samples were not analyzed for ADF and NDF in yr 1. Plasma was analyzed for Cu and Zn concentrations by flame atomic absorption spectrophotometry (model 5000, Perkin-Elmer, Norwalk, Conn.).

**Statistical Analysis.** Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, N.C.). Adherence of the data to the assumptions of the statistical test was established. Models included treatment as a fixed effect and calf and year as a random effect. Weight data were analyzed as repeated measures using the compound symmetry (CS) covariance structure. Plasma trace mineral concentrations were analyzed as repeated measures using the variance components (VC) covariance structure. Calf was the experimental unit, as treatments were delivered to individual calves. Least squares means were partitioned at the 5% level of significance by means of the probability of differences (PDIF) option. Adjustment of the level of significance was performed using the Bonferroni procedure. Statistical significance was declared at  $P < 0.05$ .

## Results and Discussion

In yr 1, an unidentified calf expelled the bolus within the first few hours after application. Reason for bolus rejection was unknown. There was no ( $P = 0.68$ , yr 1;  $P = 0.83$ , yr 2) difference in average daily gain (ADG) between bolused and non-bolused calves; however, ADG did differ between years ( $P < 0.05$ ; Table 2). The dissimilar gains observed were most likely due to greater ( $P < 0.05$ ) initial BW for yr 2 compared to yr 1, variations in herbage mass and quality between years, and implanting steers in yr 2 with Component® TE-G with Tylan®.

A freezer malfunction resulted in the loss of plasma collected from all calves on d 91 in yr 2; therefore, plasma was analyzed for Cu and Zn concentrations only among the 24 calves with blood samples collected on d 0 and 35. There were no treatment  $\times$  gender interactions for plasma mineral concentrations; thus, pooled responses are provided in Table 3. Initial plasma Cu concentrations were similar ( $P = 1.00$ , yr 1;  $P = 0.48$ , yr 2) among treatments.

Administration of the CuO bolus did not ( $P = 1.00$ , yr 1;  $P = 1.00$ , yr 2) result in greater plasma Cu concentrations compared with non-bolused calves by d 140 in yr 1 and d 35 in yr 2. Initial and final plasma Cu concentrations did not ( $P < 0.05$ , yr 1;  $P < 0.05$ , yr 2) coincide, revealing an overall decrease in plasma Cu. By the second blood collection, plasma Cu concentrations were markedly different ( $P < 0.05$ ) between yr, averaging 0.86 and 0.68 mg/L for calves in yr 1 and yr 2 respectively.

The marginal band for plasma Cu in assessing the Cu status in cattle is 0.4 to 0.7 mg/L. Thus, by d 35 calves in yr 2 the extent of Cu bioavailability was reduced by the appreciably greater S content of the DDG in yr 2; but, no apparent signs of Cu deficiency were noted during the 92-d growth trial. The maximum tolerable concentration of dietary S is estimated to be 0.5% S for roughage diets (NRC, 2005); however, this threshold was only approached by the greater S content of the DDG in yr 2. Accordingly, it is presumed that administration of the CuO bolus was ineffective and did not yield greater mean plasma Cu concentrations because DDG at 0.75% (as fed) of BW supplemented in yr 1 and yr 2 did not create abundant Cu antagonism.

Copper and Zn are absorbed through similar pathways indicating a competition for absorption sites (Oestreicher and Cousins, 1985); thus, it was necessary to determine if providing additional dietary Cu would affect plasma Zn concentrations. In this study, plasma Zn was always within adequate range. Initial and final plasma Zn concentrations coincided ( $P = 1.00$ , yr 1;  $P = 0.08$ , yr 2) among bolused and non-bolused calves, and resulting plasma Zn concentrations fell within the normal limits of 0.8 to 1.2 mg/L. The antagonism between Zn and Cu is considered a case in point of competitive interaction between minerals with similar chemical and physical properties. However, the situation regarding the reverse interaction, specifically the effects of Cu on Zn metabolism, is less clear as there is no consistent evidence that Zn absorption is seriously affected (Bremner and Beattie, 1995). Although the possibility of competitive binding of Zn transporters by added dietary Cu cannot be dismissed, similar plasma Zn concentrations among bolused and non-bolused calves casts some uncertainty on the mutuality of the antagonistic interaction.

## Implications

These results suggest that copper oxide bolus administration is ineffective in grazing beef calves that are supplemented dried distillers' grains 0.75% (as fed) of body weight. Administration of the copper oxide bolus did not impact animal performance or result in greater plasma copper concentrations, as the supplemented dried distillers' grains presumably did not create abundant copper antagonism.

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**Table 1. Dried distillers' grains (DDG) and forage nutrient composition, dry matter basis.**

Item	Year 1		Year 2	
	Hay	DDG	Forage	DDG
	----- % -----			
Crude protein <sup>a</sup>	16.6	29.6	21.5	30.2
Neutral detergent fiber <sup>a</sup>	67.5	---	48.2	43.7
Acid detergent fiber <sup>a</sup>	32.2	---	23.4	11.2
Ash <sup>a</sup>	8.9	4.5	9.1	5.3
Sulfur <sup>b</sup>	0.36	0.44	0.34	0.69
	----- mg/kg -----			
Copper <sup>b</sup>	13.50	6.13	9.40	6.16
Zinc <sup>b</sup>	52.1	82.1	41.8	79.7
Iron <sup>b</sup>	216.8	149.6	187.8	134.4

<sup>a</sup> Analyzed composition of diet.<sup>b</sup> Calculated composition of diet.**Table 2. Effect of copper oxide (CuO) bolus administration on performance of calves supplemented dried distillers' grains.**

	Heifer		Steer		SEM	Effect <sup>a</sup>
	Control	CuO bolus	Control	CuO bolus		
Final body weight, lb <sup>b</sup>						
Year 1	769	792	783	771	16.3	D
Year 2	873	856	991	989	19.6	S × D
Average daily gain, lb <sup>b</sup>						
Year 1	1.54	1.50	1.54	1.54	0.06	D
Year 2	1.79	1.73	2.33	2.42	0.07	S × D

<sup>a</sup> D = day effect, S × D = sex × day interaction,  $P < 0.05$ .<sup>b</sup> Year effect,  $P < 0.05$ .**Table 3. Effect of copper oxide (CuO) bolus administration on copper and zinc plasma concentrations of grazing calves supplemented with dried distillers' grains.**

Treatment	Plasma Copper		Plasma Zinc		SEM
	Control	CuO bolus	Control	CuO bolus	
	----- Year 1 -----				
d 0, mg/L	0.89 <sup>a</sup>	0.87 <sup>a</sup>	1.21	1.18	0.03
d 140, mg/L	0.72 <sup>b</sup>	0.71 <sup>b</sup>	1.28	1.17	0.03
	----- Year 2 -----				
d 0, mg/L	0.83 <sup>a</sup>	0.77 <sup>a</sup>	0.87	0.86	0.04
d 35, mg/L	0.68 <sup>b</sup>	0.68 <sup>b</sup>	0.94	0.96	0.04

<sup>ab</sup> Within a year and column, least square means with different superscripts differ ( $P < 0.05$ ).

# Effects of feed delivery methods for stocker calves grazing bermudagrass on growth performance, behavior, and labor inputs<sup>1</sup>

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## Story in Brief

The successes of new technology, such as automated feeders (Solar Feeders, Inc., Fort Smith, Ark.) have not been explored as a potential resource for supplementing grazing stocker calves. Crossbred heifers (n = 78, initial body weight = 541 ± 7.7 lb) were used to evaluate the effects of delivery methods on behavior, body weight gain, pasture and corn gluten supplement utilization, and labor inputs. Calves were stratified by body weight and assigned randomly to 1 of 6, 6-acre bermudagrass pastures. Pastures were blocked by location and assigned randomly to 1 of 3 delivery treatments: 1) hand feeding corn gluten once daily; 2) automated feeder dispersing corn gluten 3 times/d; or 3) corn gluten mixed with 7.5% salt offered ad-libitum. Corn gluten was offered at 1% then increased to 1.5% of body weight. Data were analyzed using pen as the experimental unit. For the 85-day trial, cattle on all treatments had similar final body weights ( $P = 0.22$ ). Hand fed heifers had greater ( $P = 0.05$ ) average daily gain at day 28 than heifers fed with the automated feeder and the salt limited supplement. Total average daily gain (day 0 to 85) tended to be lower in salt limited calves compared to hand fed (1.5 vs. 1.8 lb;  $P = 0.08$ ). Data from accelerometers (HoBeware Pendant G, Onset Computer Corp, Bourne, Mass.) attached to 3 heifers/pasture for 7-day periods at 14 day intervals indicated that automated feeder-calves spent a greater proportion of time standing ( $P < 0.05$ ). The automated feeder required less ( $P < 0.05$ ) feeding labor (30 min/wk) than hand fed (43 min/wk) or salt limited (41 min/wk). Results indicated that using the automated feeder in place of hand feeding or using a salt limited feed had minimal effects on growth performance or behavior while saving the producer labor.

## Introduction

In providing supplemental feed to growing cattle, today's producers are faced with challenges. These include off-farm job responsibilities restricting the amount of time available to deliver feed, farm expansion often requires purchasing property not adjacent to the headquarters location, and growing input costs including fuel cost for delivering feed. To combat these challenges, producers have utilized alternate day feeding or feed additives such as salt for self-fed programs. Both of these solutions have drawbacks. Alternate day feeding is successful at low supplementation rates but may affect diet digestibility and performance at greater rates. Salt is hard on equipment due to its corrosive nature and some cattle may not adapt to salt limited supplements which increases variability in growth response. An alternative would be to utilize an automated supplement delivery system, supporting daily supplementation when supplementation rates were greater than acceptable levels for alternate day supplementation. Plus, automated feeding would not require corrosive salt and also provide a supplement that would be more uniformly accepted among cattle. Therefore, the objective of this study was to examine performance and behavioral changes in stocker calves on summer pasture as affected by self-fed supplements controlled by salt or automated feed delivery.

## Materials and Methods

Two automated feeders (Fig. 1; Auto Feeder 2500, Solar Feeders, Inc., Fort Smith, Ark.) were delivered to the University of Arkansas Division of Agriculture Stocker and Receiving Cattle Facility. The same corn gluten feed that was to be used to supplement the cattle was used to calibrate the feeders. A feed delivery prediction equation was established by measuring the amount of feed expressed by

varying spinner time "ON" and distance from spinner to hopper, the 2 points of adjustment on each feeder. These equations were used to adjust the amount of feed that the automated feeders delivered through the project.

Seventy-eight preconditioned heifers (initial body weight = 541 lb) were obtained from a cooperating producer. Cattle had been previously vaccinated against clostridial diseases and respiratory viruses. Cattle were identified with a unique individual ear tag and were given an anthelmintic before the project began. Cattle were weighed on d -7 and -6 (June 5 and 6, 2012), an average was calculated and then heifers were stratified by this body weight and assigned randomly to 1 of 6 pastures, such that the average body weight of cattle in each pasture were similar. Pastures were 6 acres each, contained predominately bermudagrass, and water was available for ad libitum consumption via automated waterers. Pastures were blocked by location, and within each block pastures were assigned randomly to 1 of the 3 treatments.

Treatments were: 1) control – corn gluten feed hand fed 1 time each day (7:30 a.m.) at a rate of 1% of body weight, fed in a single 20 ft bunk in each pasture; 2) salt-limited corn gluten feed free choice delivering supplement at 1% of body weight with an expected salt intake of 0.1 lb/100 lb body weight, this was offered in a wooden feeder, that's outer surfaces were covered with metal sheeting, with 8 ft of bunk space on each of 2 sides (16 ft total bunk space); and 3) corn gluten feed delivered by an automated feeder 3 times each day (6:00 a.m., 2:00 p.m., and 10:00 p.m.) to total a rate of 1% of body weight, the automated feeders had 27 ft of bunk space. A mineral supplement (Pasture Mineral + Mag, Tri State Agri Services, Afton, Okla.) was mixed into the salt-limited mix, and was offered as a free choice mineral supplement to the cattle on the other treatments. Both the salt limited and automated feeders were filled with a 7 d supply of feed. The salt content of the salt-limiting supplement was

<sup>1</sup> This project was partially funded by Solar Feeders, Inc. Fort Smith, Ark.; their support is gratefully acknowledged.

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modified during the project when desired intake was not occurring. Initially, the mixture was 9.1% salt (8.18% white salt and 3.32% mineral supplement [Pasture Mineral + Mag]). On d 35 the mixture was changed to 7.5% salt. Corn gluten feed and salts were added to a Knight mixer wagon (Kuhn North America, Inc., Brodhead, Wisc.) and allowed to mix for 10 min before being dumped onto a covered concrete slab for storage until fed.

Cattle were sorted into groups on d 6 and moved to the appropriate pastures where all cattle were offered corn gluten feed by hand feeding in the bunks that would be used for each pasture during the remainder of the study. On d 0, cattle were weighed and then the automated feeders were turned on and the salt limited ration was offered. On d 28, it was determined that there was going to be a lack of available forage through the study due to drought conditions. At that time, the rate of supplementation of corn gluten feed for all 3 treatments was increased to 1.5% of body weight.

Calf body weight was measured on d 7, 6, 0, 7, 28, 56, 84, and 85. Pasture forage mass was measured on d 7, 23, and 49 by calibrated disk meter, and pasture grab samples were taken for nutrient analysis on d 7, 23, 49, and 75. Although the intent had been to measure pasture forage mass near the end of the project, this was not done due to the lack of pasture forage mass at that time. Beginning on d 56, bermudagrass hay was offered in large round bales for ad libitum consumption, samples of the hay were obtained for nutrient analysis.

Feed delivery and estimated consumption were determined every 7 d by weighing back any unused feed and recording the amount of feed added to the feeders. Fresh and unused feed were sampled for dry matter determination, and feed disappearance was corrected for any dry matter differences. Weights of fed and refused salt limited supplement were also corrected for the salt content. All feedstuffs (pasture, hay, and corn gluten) were tested for nutrient composition (dry matter, crude protein, ash, neutral detergent fiber, acid detergent fiber, and sodium). Time spent feeding within each pasture was recorded, this being the time spent filling or maintaining the feed bunks in each pasture. A log was also kept of the man-hours needed to prepare the supplement for delivery; for the hand fed and automated feeder methods, this was the time spent filling, weighing, and loading buckets of corn gluten onto a truck for delivery either daily (hand fed) or weekly (automated feeder). For the salt limiting supplement, the preparation time also included mixing the corn gluten feed with salt. Due to some missing data in these logs, the project was divided into 5 periods (approximately 2 wk in length) and weekly averages were calculated for each period. The time spent traveling from the feed storage location to each pasture was not recorded.

Calf behavior during daylight hours was monitored visually every 14 d; the number of calves within each pen exhibiting the following behaviors (lying, standing idle, walking, drinking, or eating [supplement or hay]) was recorded. Visual observations were taken on an hourly basis from 6:00 to 9:00 a.m., from noon to 3:00 p.m., and from 6:00 to 8:00 p.m.

Beginning on d 0, 28, 56, and 77, 3 calves/pasture were fitted with a data-logging accelerometer attached to a leg for 7 d. Calf standing and lying activity were monitored by recording x- and y-axis positions of the accelerometer. This gave information on the amount of time spent resting vs. standing (i.e. grazing). The same calves were used throughout the project.

Data were analyzed using the mixed procedures of SAS (SAS Institute, Inc., Cary, N.C.). The experimental unit was pen. The model included the fixed effect of treatment and where appropriate day (or period), and the 2-way interaction. Block (pasture loca-

tion) was a random effect. Kenward Rogers was specified as the degrees of freedom selection method. When appropriate, the repeated statement was used with a spatial power structure. For the accelerometer data, an R statistical software cluster analysis was done for each calf using the clara function and combined total counts from 3 identified clusters. This data was analyzed using the general linear models procedure with treatment as the fixed effect and number of events of the dependent variable. The response was a binomial distribution and the logit link function was applied.

## Results and Discussion

Supplement and forage nutrient analyses are shown in Table 1. Pasture forage mass was not impacted by the method of supplement delivery ( $P = 0.98$ , Fig. 2). There was a dramatic decrease in pasture forage mass as the study progressed (day,  $P < 0.0001$ ). The summer was extremely dry, less than 4.7 in of rain fell during the study resulting in a 6 in deficit in precipitation for these months. Maximum daily temperatures averaged 94 °F during the trial. Due to this weather event, the supplementation rate with corn gluten was increased from 1% to 1.5% of body weight on d 28; and beginning on d 56, bermudagrass hay was offered.

Heifer body weight did not differ ( $P > 0.17$ , Fig. 3) at any time during the study. Heifer average daily gain (ADG; Table 2) was affected by supplement delivery method from d 0 to 28; hand fed heifers had a greater ( $P = 0.05$ ) ADG during this first period than heifers fed with the automated feeder and the salt limited supplement. However there were no effects of method of supplement delivery on ADG during any other periods. For the entire 85 d trial, hand fed heifers tended to have a greater ( $P = 0.08$ ) ADG than heifers offered the salt limited supplement and the heifers fed with the automated feeder were intermediate. Supplement disappearance was affected by a treatment  $\times$  period interaction ( $P < 0.001$ , Table 2). Cattle on all treatments consumed the same amount of corn gluten feed from d 0 to 56. However, during the last period the cattle consuming the salt limited ration did not meet their target supplement consumption. The automated feeders consistently delivered the expected amount of supplement to the cattle throughout the 85 d study. The partial feed conversion, pounds of corn gluten fed/pound of weight gain (Table 2), was not affected ( $P = 0.63$ ) by delivery method. Partial feed conversion was affected by period ( $P = 0.05$ ), as the summer progressed and forage quality changed calves consumed more corn gluten for each pound gained.

There were no differences ( $P > 0.14$ ) detected through the biweekly visual observations in the amount of time heifers spent laying, standing, grazing, or mobile due to supplement delivery method. The accelerometer data indicated that heifers fed with the automated feeders spent more time standing ( $P < 0.05$ , data not shown).

Labor hours (Table 3) were divided into preparation and feeding times, as well as the combined overall time. There was a treatment  $\times$  period interaction ( $P < 0.01$ ) for preparation time. The salt limited supplement had a greater preparation time than the other delivery methods during periods when it was mixed. There was also a main effect of treatment ( $P < 0.01$ ); it took longer to prepare the salt limited supplement than to prepare feed for the other feeding methods. There was not a treatment  $\times$  period interaction ( $P = 0.34$ ) for time spent feeding. Time spent feeding differed among treatments ( $P = 0.002$ ), hand feeding took the longest time, the shortest time was required to feed using the automated feeders, and feeding the salt limited supplement took an intermediate amount of time. The automated feeders ran as expected through the 85 d



project and required no maintenance. There was also a period effect ( $P = 0.02$ ) on feeding time, as the study progressed (and cattle were consuming more supplement) it took longer to deliver supplement. In this project, workers brought the supplement in buckets to the automated feeder as it sat in the pasture. It should be noted that these feeders are made with the capability to be moved to obtain feed, or alternatively a truck delivering bulk feed could go to the feeder. Either of those alternatives would impact the labor needed to use an automated feeder in each producer's unique situation. When preparation and feeding times were combined into total man-hours, using the automated feeders took less time ( $P = 0.04$ )

than both hand feeding and using a salt limited supplement which did not differ in man-hours required.

## Implications

Overall there were minimal changes in animal gain or behavior among supplement delivery methods. Increased labor was required for preparing the salt limited supplement and feeding the hand fed supplement. Automated feeders can be an alternative supplement delivery option for producers that are short-staffed or have cattle located over broad geographic locations.

**Table 1. Nutrient composition of feeds (dry matter basis).**

	CP (%)	NDF (%)	ADF (%)	Ash (%)	Na (%)
Corn gluten feed	23.2	39.5	9.3	11.1	0.24
Salt limited corn gluten feed <sup>†</sup>	21.6	34.7	8.0	19.81	2.10
Salt limited corn gluten feed <sup>‡</sup>	20.9	38.1	8.4	17.94	1.81
Pasture forage					
June 5	15.0	68.7	32.8	8.02	0.01
July 5	10.8	63.0	28.4	8.92	0.01
July 31	10.9	68.8	33.8	6.63	0.01
August 26	10.4	73.0	31.0	6.42	0.03
Bermudagrass hay	11.1	73.9	32.0	6.55	0.05

<sup>†</sup>Corn gluten feed mixed with 9.1% white salt.

<sup>‡</sup>Corn gluten feed mixed with 7.5% white salt.

**Table 2. Effects of feed delivery methods for stocker calves on average daily gain, corn gluten feed disappearance, and supplement use efficiency.**

	Automated feeder	Hand fed	Salt limited	SE	P-value
Average daily gain, lb					
Day 0 to 28	1.95 <sup>b</sup>	2.29 <sup>a</sup>	1.99 <sup>b</sup>	0.058	0.05
Day 29 to 56	1.90	1.76	2.06	0.271	0.72
Day 57 to 85	0.98	1.36	0.55	0.277	0.26
Day 0 to 85	1.59 <sup>cd</sup>	1.78 <sup>c</sup>	1.52 <sup>d</sup>	0.051	0.08
Corn gluten feed disappearance, lb/day <sup>†</sup>					
Day 0 to 28	5.0	5.2	4.9	0.53	--
Day 29 to 56	8.8	8.5	7.0	0.53	--
Day 57 to 85	8.9 <sup>a</sup>	9.4 <sup>a</sup>	6.3 <sup>b</sup>	0.53	--
Day 0 to 85	7.6	7.7	6.1	0.52	0.20
Partial feed conversion (corn gluten feed intake, lb/weight gain, lb) <sup>‡</sup>					
Day 0 to 28	2.6	2.3	2.5	2.14	--
Day 29 to 56	4.7	4.9	3.4	2.14	--
Day 57 to 85	9.2	7.6	13.4	2.14	--
Day 0 to 85	5.5	4.9	6.4	1.03	0.57

<sup>†</sup> Period,  $P < 0.0001$ ; treatment  $\times$  period,  $P = 0.0003$ .

<sup>‡</sup> Period,  $P = 0.05$

<sup>ab</sup> Least-squares means within a row with differing superscripts differ ( $P < 0.05$ ).

<sup>cd</sup> Least-squares means within a row with differing superscripts differ ( $P < 0.05$ ), with an F-test of  $P = 0.08$ .

SE = standard error.

**Table 3. Effects of feed delivery methods for stocker calves on time spent each week preparing and delivering feed.**

	Automated feeder	Hand fed	Salt limited	SE
Preparation time, min/week <sup>†</sup>				
Period 1	12.1 <sup>b</sup>	5.0 <sup>b</sup>	37.9 <sup>a</sup>	3.3
Period 2	22.0 <sup>a</sup>	13.2 <sup>b</sup>	23.2 <sup>a</sup>	3.3
Period 3	23.6 <sup>b</sup>	18.1 <sup>b</sup>	32.0 <sup>a</sup>	3.3
Period 4	23.5	25.7	24.1	3.3
Period 5	21.9 <sup>ab</sup>	27.7 <sup>a</sup>	15.3 <sup>b</sup>	3.3
Overall	20.6 <sup>b</sup>	17.9 <sup>b</sup>	26.5 <sup>a</sup>	0.97
Delivery time, min/week <sup>‡</sup>				
Overall	9.3 <sup>c</sup>	24.7 <sup>a</sup>	14.2 <sup>b</sup>	0.82
Time spent feeding, min/week <sup>§</sup>				
Period 1	18.6 <sup>b</sup>	23.6 <sup>b</sup>	48.9 <sup>a</sup>	3.1
Period 2	31.8 <sup>b</sup>	41.8 <sup>a</sup>	35.5 <sup>ab</sup>	3.1
Period 3	32.9 <sup>b</sup>	49.3 <sup>a</sup>	51.3 <sup>a</sup>	3.1
Period 4	37.1 <sup>b</sup>	49.0 <sup>a</sup>	42.2 <sup>ab</sup>	3.1
Period 5	29.4 <sup>b</sup>	49.4 <sup>a</sup>	25.7 <sup>b</sup>	3.1
Overall	30.0 <sup>b</sup>	42.6 <sup>a</sup>	40.7 <sup>a</sup>	2.1

<sup>†</sup> Treatment,  $P = 0.002$ ; treatment  $\times$  period,  $P = 0.002$ .

<sup>‡</sup> Treatment,  $P = 0.002$ .

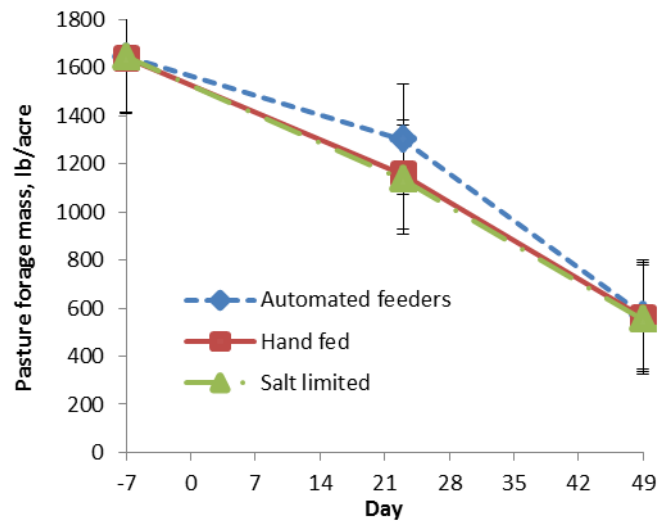
<sup>§</sup> Treatment,  $P = 0.04$ , Treatment  $\times$  period,  $P < 0.0001$ .

<sup>abc</sup> Least-squares means within a row with differing superscripts differ ( $P < 0.05$ ).

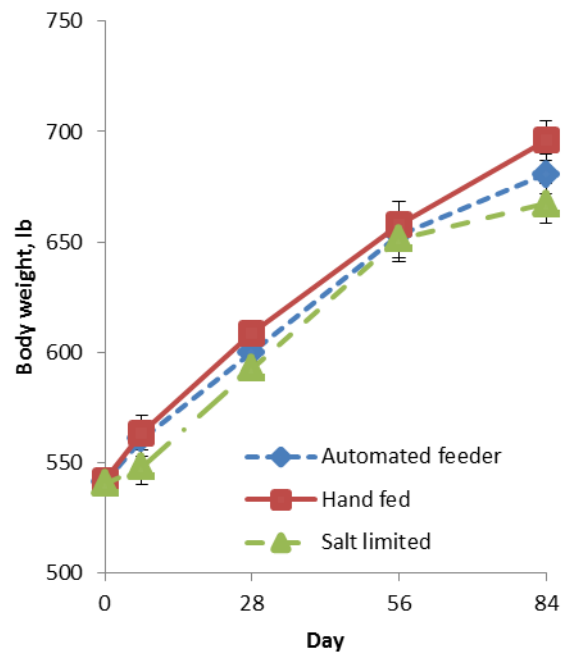
SE = standard error.



**Fig. 1. Automated feeder (Auto Feeder 2500, Solar Feeders, Inc., Fort Smith, Ark.).**



**Fig. 2.** Effects of feed delivery methods for stocker calves on pasture forage mass. Day,  $P < 0.001$ .



**Fig. 3.** Effects of feed delivery methods for stocker calves on body weight. Treatment,  $P > 0.17$ .

# Horn fly and sire breed affects on milk production traits of beef cows<sup>1</sup>

A.R. Mays<sup>2</sup>, M.A. Brown<sup>3</sup> and C.F. Rosenkrans, Jr.<sup>2</sup>

## Story in Brief

Horn fly infestations can negatively impact profitability traits of beef cattle which has lead to a variety of strategies to minimize the effects of flies on cattle productivity. Our objective was to determine the relationship between sire breed on horn fly infestation, and milk quality and quantity of beef cows (n = 53). All cows had Brangus dams and were sired by Bonsmara (BONS; n = 7), Brangus (BRAN; n = 13), Charolais (CHAR; n = 8), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 12), or Romosinuano (ROMO; n = 8) bulls. Horn fly counts varied by month ( $P < 0.0001$ ), with the lowest population recorded in May ( $99 \pm 39$  flies) and peaking in August ( $520 \pm 38$  flies). An effect of sire breed  $\times$  log horn fly count affected ( $P < 0.05$ ) milk yield, as well as an interaction of prolactin  $\times$  sire breed ( $P < 0.10$ ). Our results indicate horn fly infestation negatively impacts milk yield and quality traits of beef cows. Horn fly numbers also are influenced by sire breed and period of lactation cycle. Future multi-trait selection schemes for beef cattle may include horn fly resistance for improved sustainability.

## Introduction

Horn flies (*Haematobia irritans*) are often described as pasture insects, with cattle being the primary host (Williams et al., 1985). In North America, horn flies are regularly observed clustered on the backs of cattle, which provide an ideal location for horn flies to feed. Costs attributed to horn flies have been reported to equal \$876 million (Kunz et al., 1991). With increased resistance to pesticides and increased value of cattle, it is reasonable to assume production losses due to horn flies have increased.

Data addressing the effects of horn flies on milk production traits of beef cattle is limited and inconsistent. Milk yield has been reported to increase in dairy cows treated for horn flies (Block and Lewis, 1986), while other studies reported no effect on milk yield in dairy cows (Cheng and Kessler, 1961; Miller et al., 1973). Therefore, our objective was to determine the effects of horn flies on milk production quantity and quality traits of beef cows.

## Materials and Methods

The Committee for Animal Welfare at the USDA-ARS, Grazinglands Research Laboratory El Reno, Okla., and the University of Arkansas Institutional Animal Care and Use Committee approved the procedures used in this study.

**Horn Fly Counts.** Total horn fly populations were recorded on individual animals, while in their pasture from 0700 through 0900 hours, every 28 days beginning in May and ending in October. Individual animals were observed by a trained individual throughout the study, utilizing binoculars for accurate counts if animals were greater than 5 meters away. After horn fly counts were recorded, horn flies were treated using Co-Ral (organophosphate; Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, Kan.), when populations exceeded threshold levels ( $>200$  flies/animal).

**Milk Production.** Estimates of milk yield and quality were collected from cows that had Brangus dams and were sired by Bonsmara (BONS; n = 7), Brangus (BRAN; n = 13), Charolais (CHAR; n = 8), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 12), or Romosinuano (ROMO; n = 8) bulls. A single-cow portable milking ma-

chine was utilized to milk cows. Milk yield was assessed every 28 days beginning in late May and ending in late October.

Ten minutes prior to milking, cows were administered 1.5 mL of acepromazine maleate (10 mg/mL, i.m.), and 1.0 mL of oxytocin (20 USP units/mL) was given immediately before milking to facilitate milk letdown. Milk was weighed on a digital platform scale and adjusted to a 24 h basis [(milk weight/14)  $\times$  24]. A commercial dairy laboratory was responsible for milk quality analysis, which included estimates of milk fat, protein, urea nitrogen, somatic cell count (SCC), lactose, and solids-not-fat (SNF).

**Blood Serum Hormone Analysis.** Blood samples were collected monthly, beginning in May and ending in October, via jugular venipuncture using vacutainers (Bectin Dickinson, Franklin Lakes, N.J.). Samples were allowed to clot for 24 h at 4 °C, and centrifuged at 2,500  $\times$  g for 25 min (minute) at 4 °C (Marathon 22KBR, Fisher Scientific, Hermle-Labortechnik, Germany). Serum was then harvested and stored at -20 °C pending analysis.

Prolactin concentrations were analyzed in duplicate by double-antibody RIA using primary antisera, purified standard, and iodination preparations supplied by the National Hormone and Peptide Program (Torrance, Calif.).

**Statistical Analysis.** Horn fly counts were transformed to natural log horn fly count prior to analysis. Data for milk yield, milk quality, and horn fly count were analyzed by mixed model least squares (SAS Institute, Cary, N.C.) using a linear model that included sire breed (fixed), cow nested in sire breed (random), month (fixed repeated), and month  $\times$  sire breed, with calf birth date as a linear covariate. Calf birth date was not a significant covariate and was dropped from the model. Effects of horn fly count on milk yield and quality were estimated by including a linear covariate of log horn fly count (linear) and log horn fly count  $\times$  sire breed.

The analysis of prolactin concentration (PRL) regression on log horn fly count used mixed model least squares, with a linear model of sire breed (fixed), cow nested in sire breed (random), month (fixed repeated), month  $\times$  sire breed, log horn fly count (linear), and log horn fly count  $\times$  sire breed. Prolactin data also was analyzed as the regression of milk yield on PRL with the full linear model including sire breed (fixed), cow nested in sire breed (random),

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<sup>3</sup> USDA-ARS, Grazinglands Research Laboratory, El Reno, Oklahoma.

month (fixed repeated), month  $\times$  sire breed, PRL (linear), and PRL  $\times$  sire breed. All models used were reduced in a step-wise procedure by elimination of insignificant interactions ( $P > 0.25$ ) and in accordance with appropriate model reduction procedures.

## Results and Discussion

**Horn Flies.** Horn fly counts varied by month ( $P < 0.0001$ ), with the lowest population recorded in May ( $99 \pm 39$  flies) and populations peaking in August ( $520 \pm 38$  flies; Fig. 1). However, sire breed differences ( $P > 0.25$ ; Fig. 2) and a sire breed  $\times$  month interaction ( $P > 0.10$ ) did not occur in this study.

**Milk Yield.** Milk yield was affected ( $P < 0.05$ ) by sire breed (Fig. 3). Bonsmara and GELV sired cows had increased milk yield when compared to HERF sired cows ( $8.75 \pm 0.73$  and  $8.62 \pm 0.86$  versus  $6.02 \pm 0.57$  kg/d; respectively), with CHAR, ROMO and BRAN sired cows intermediate ( $7.28 \pm 0.65$ ,  $7.00 \pm 0.65$ , and  $7.06 \pm 0.56$  kg/d; respectively).

An interaction of sire breed and log horn fly count affected ( $P < 0.05$ ) milk yield (Fig. 4). Milk yield was reduced by 0.99 and 0.64 kg/d per unit increase in log horn fly count in GELV and BONS sired cows. There was less evidence of horn fly count effects on milk yield in other sire breeds ( $P > 0.25$ ). However, the regression coefficients for other sire breeds were negative, with the exception of BRAN sired cows. The regression coefficient for milk yield on log horn fly count was less in GELV sired cows than BRAN, CHAR, HERF, and ROMO sired cows ( $P < 0.01$ ), and less in BONS sired cows than BRAN sired cows ( $P < 0.05$ ), where less indicates greater reductions in milk yield (Table 1).

A month by log horn fly count interaction also affected ( $P < 0.05$ ) milk yield (Fig. 5). Milk yield was reduced by 0.72, 0.68, and 0.71 kg/d per unit increase in log horn fly count in May, June and July. However, the regression coefficients for milk yield in August and September were positive, while the regression coefficient for October was negative.

**Milk Quality.** Milk lactose and somatic cell count (SCC) were not affected ( $P > 0.25$ ) by horn flies. However, for every one unit increase in log horn fly count, percent milk fat decreased by 0.15% ( $P < 0.05$ ), percent solids-non-fat (SNF) decreased by 0.10% ( $P < 0.05$ ), and milk urea nitrogen decreased by 0.62 mg/dL ( $P < 0.02$ ). In HERF sired cows, percent milk protein decreased by 0.15% ( $P < 0.01$ ) per unit increase in log horn fly count, while percent milk protein of other sire breeds was not affected.

**Serum Prolactin.** The regression of log horn fly count on PRL was not affected ( $P > 0.25$ ) by sire breed. An interaction of PRL and sire breed affected ( $P < 0.10$ ) milk yield (Fig. 6). The regression coefficients for all sire breeds except BONS and GELV sired cows were negative, indicating a reduction in milk yield.

Variation among breed types and horn fly populations have been reported in the literature, as well as variation among breed types and milk production traits. However, the evaluation of breed type and horn flies, and their impact on milk production in beef cows, is lacking. Unlike other studies, our results indicated horn fly populations did not differ between sire breeds. Therefore, a combination of sire breed and effects of horn flies may provide a better understanding of the impact these variables have on beef cow milk production.

Milk yield was affected by sire breed, but these results differ from those of Brown and Lalman (2010) who reported ROMO sired cows to have decreased milk yield compared to BONS, BRAN, CHAR, GELV, and HERF sired cows. However, when the same sire breeds were assessed in our study, BONS sired cows numerically

had the greatest milk yield compared to other sire breeds, but were statistically similar to GELV, CHAR and ROMO. However, the current study was based on one lactation cycle; whereas, the results of Brown and Lalman (2010) were based on multiple years.

The interaction between horn flies and sire breed also contributed to milk yield differences observed in this study. Milk yield was negatively impacted by horn flies in BONS and GELV sired cows compared to other sire breeds. Perhaps the greater milk yield observed for these two sire breeds was more negatively impacted by the induced stress caused by horn flies. Hereford sired cows produced the lowest milk yield of all sire breeds, but were not as negatively impacted by horn flies. Although our results indicate horn flies negatively impact milk yield, and this impact is dependent upon sire breed results from other studies, reporting horn flies may or may not affect milk yield of cows treated or not treated for horn flies (Cheng and Kessler, 1961; Miller et. al., 1973; Block and Lewis, 1986).

Horn flies also negatively impacted milk quality traits, including milk fat, SNF, and urea nitrogen. However, percent milk protein was only affected by horn flies in HERF sired cows. Block and Lewis (1986) reported milk fat and protein percentage were not affected ( $P > 0.05$ ) by horn fly treatment or lack of treatment in Holstein cows.

Our study also evaluated the relationship between milk yield and serum PRL concentrations. Milk yield of GELV and BONS sired cows was not negatively affected by this relationship. Therefore, milk yield may be affected differently based on both sire breed and the synthesis and release of serum PRL, which varies among individual animals.

An additional variable beyond sire breed to consider is period of lactation, as horn flies had a greater impact on milk production at the beginning of lactation. Initiating this cycle is stressful for the cow, having recently given birth and preparing to breed back. In combination with the stress of beginning the lactation cycle, the additional stress induced horn flies may explain the observed reduction in milk yield.

## Implications

Potential explanations for the variation between our results and other studies may be due to their utilization of dairy cattle breeds instead of beef, environmental differences, or geographic location. Further research regarding the effects of horn flies on milk production traits of beef cows is needed to confirm and elaborate on our findings. More breed types, geographic locations, and variation in management practices should also be taken into account in future research.

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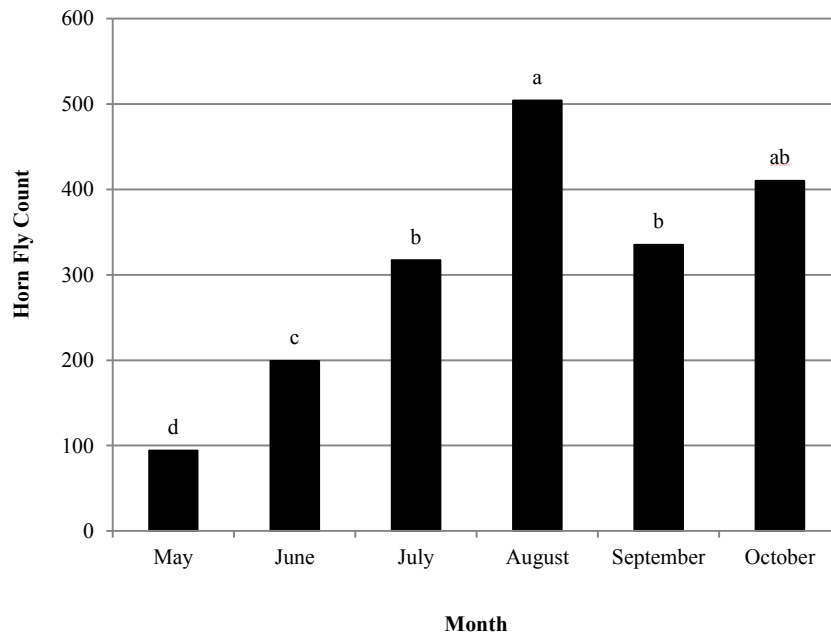
Williams, R. E., R. D. Hall, A. B. Broce, and P. J. Scholl. 1985. *Livestock Entomology.* John Wiley and Sons, Inc. New York, N.Y.

**Table 1. Regression Coefficients and Standard Errors of Milk Yield on Log Horn Fly count by Sire Breed.**

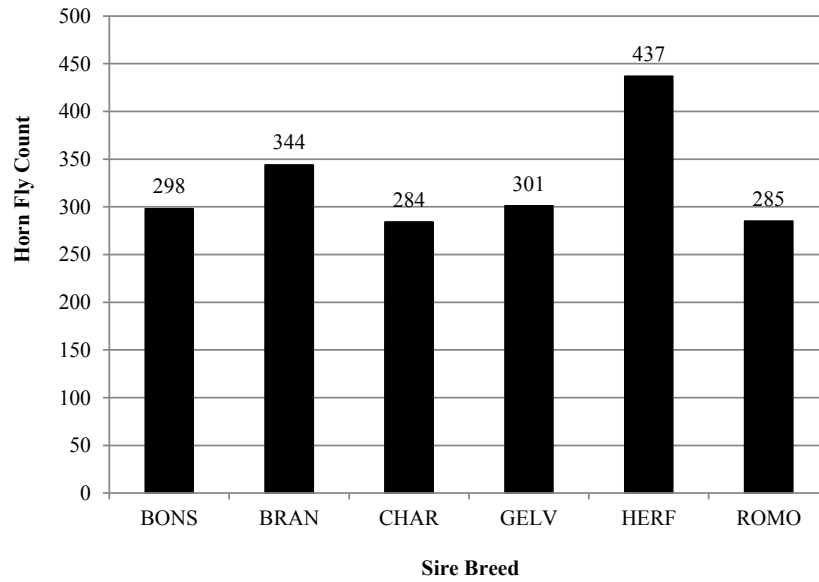
	Sire Breed					
	BONS	BRAN	CHAR	GELV	HERF	ROMO
Estimate	-0.64 <sup>ab</sup>	0.30 <sup>c</sup>	-0.026 <sup>bc</sup>	-0.99 <sup>a</sup>	-0.17 <sup>bc</sup>	-0.16 <sup>bc</sup>
SE	0.30	0.26	0.22	0.32	0.20	0.17

<sup>a,b,c,d</sup> Values without common superscripts differ ( $P < 0.05$ ).

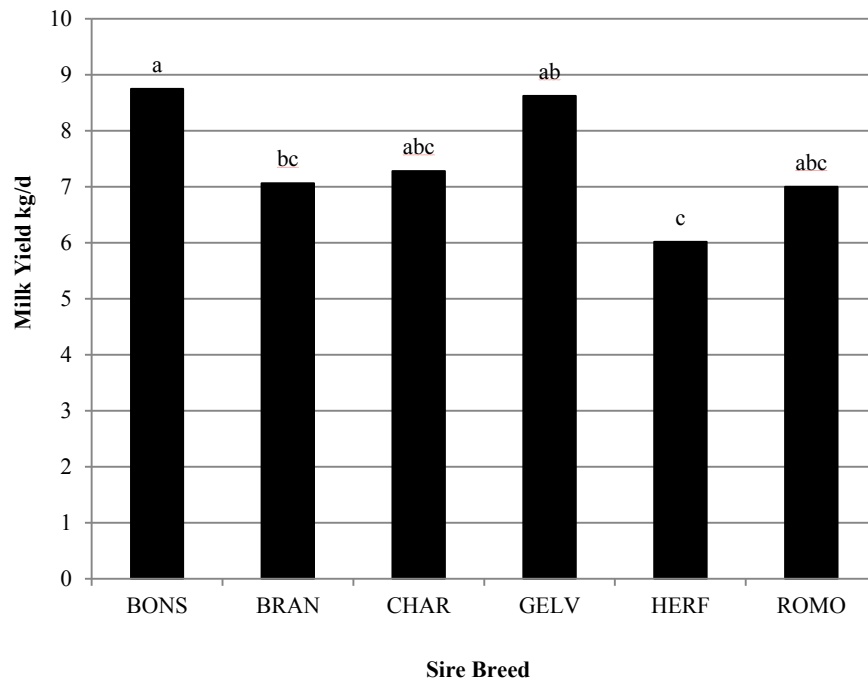
SE = Standard Errors, BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.



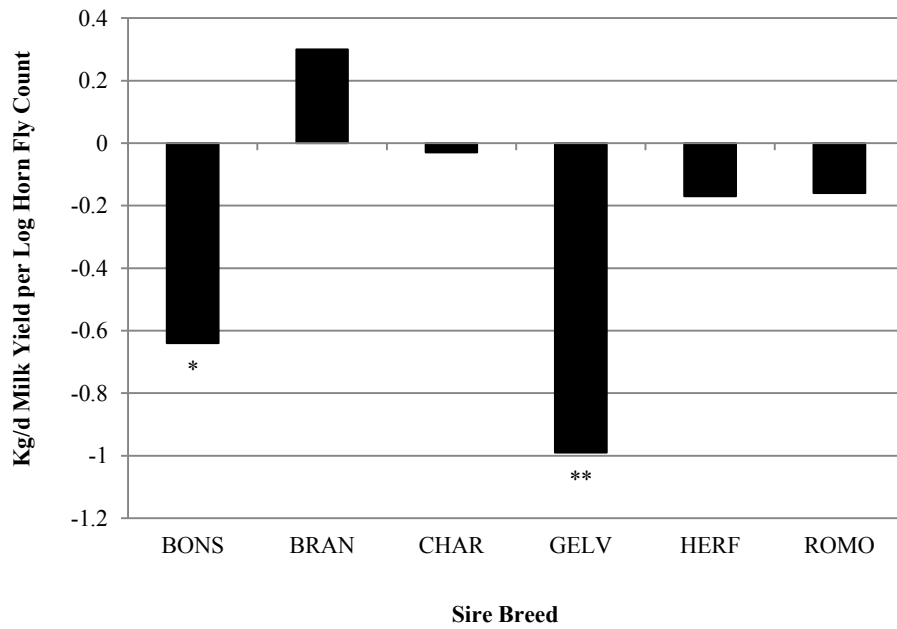
**Fig. 1. Monthly Horn Fly Count. ( $P < 0.0001$ ; SEM = 37.87). SEM = Standard Error Means. <sup>a,b,c,d</sup> Bars without common superscripts differ.**



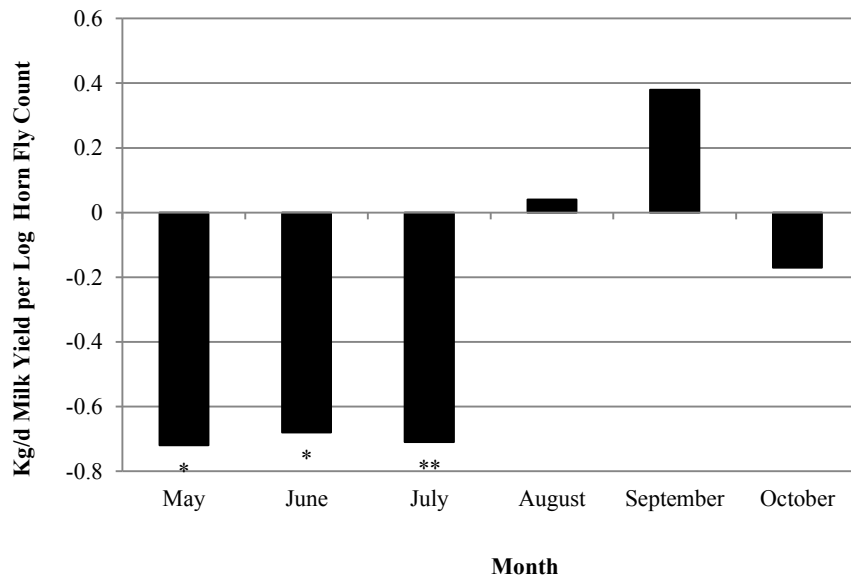
**Fig. 2. Horn Fly Counts of Sire Breeds. ( $P > 0.25$ ; EM = 59.42). SEM = Standard Error Means. BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.**



**Fig. 3. Average Milk Yield of Sire Breeds. ( $P < 0.05$ ; SEM = 0.61). SEM = Standard Error Means. <sup>a,b,c</sup> Bars without common superscripts differ. BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.**

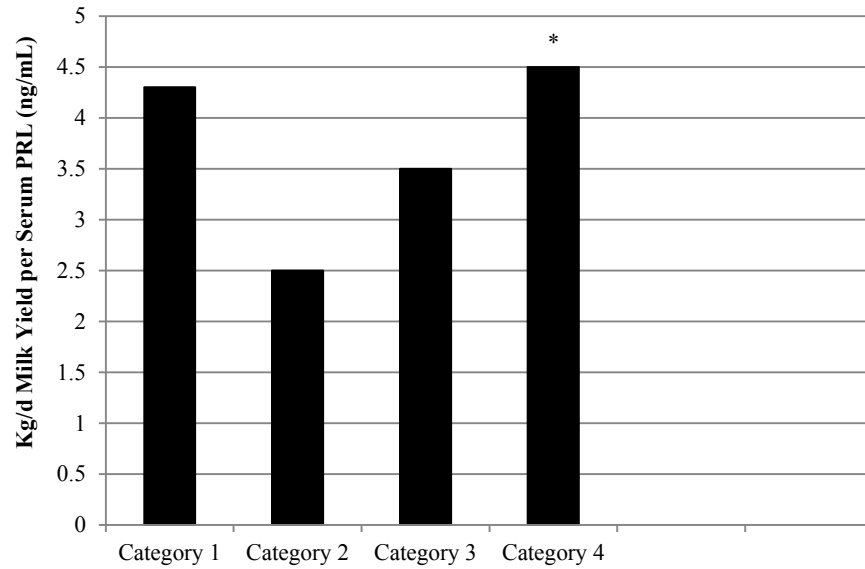


**Fig. 4. Regression of Milk Yield on Log Horn Fly Count by Sire Breed. (\*\* $P < 0.01$ ; \* $P < 0.05$ ; SEM = 0.25). SEM = Standard Error Means. BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.**



**Fig. 5. Regression of Milk Yield on Log Horn Fly Count by Month. (\*\* $P < 0.001$ ; \* $P < 0.05$ ; SEM = 0.32). SEM = Standard Error Means.**





**Fig. 6. Regression of Milk Yield on Serum Prolactin by Sire Breed. (\* $P < 0.05$ ; SEM = 0.004). SEM = Standard Error Means, PRL = Prolactin concentrations (ng/mL), BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.**

# Association of genotypes from the promoter region of the bovine prolactin gene with milk production traits and horn fly resistance of beef cows<sup>1</sup>

A.R. Mays<sup>2</sup>, M.A. Brown<sup>3</sup> and C.F. Rosenkrans, Jr.<sup>2</sup>

## Story in Brief

Genetic selection for increased milk production and horn fly resistance may be useful for improving management of beef cows. The objective of this study was to categorize cows using a single nucleotide polymorphism in the promoter region of the prolactin gene (C1286T). The primary focus was to determine any association of the single nucleotide polymorphism with milk production and horn fly resistance. All three genotypes of the polymorphism from the promoter region of the prolactin gene, homozygous cytosine (CC), thymine (TT), and heterozygous (CT), were observed in the study population. Prolactin genotypes were not associated with milk quantity, quality, or horn fly resistance in this study.

## Introduction

Single nucleotide polymorphisms (SNP) are mutations in DNA that can result in animals with different phenotypes and production characteristics (Sylvänen, 2001). Profitability traits in cattle have been associated with SNP within the promoter region of the prolactin (PRL) gene. Milk quality and quantity from dairy cows was associated with mutation in the promoter and coding sequence of the PRL gene (Brym et al., 2005; Lü et al., 2010; Nasrin et al., 2009)

Associations between cattle genotype and horn fly resistance have not been reported; however, we have previously demonstrated that horn fly resistance had a moderate heritability coefficient (Brown et al., 1992). Our objective was to evaluate the association of PRL genotypes, milk production, and horn fly density.

## Materials and Methods

The Animal Care and Use Committees of the USDA-ARS Grazinglands Research Laboratory El Reno, Okla., the University of Arkansas and The Oklahoma State University approved the procedures used in this study.

**Milk Production.** Estimates of milk yield and quality were collected from Bonsmara (BONS; n = 7), Brangus (BRAN; n = 13), Charolais (CHAR; n = 8), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 12), and Romosinuano (ROMO; n = 8) sired cows. A single-cow portable milking machine was utilized to measure milk yield beginning 60 d postpartum. Measurements of milk yield began in late May and ended in late October, and were collected every 28 d during this time period.

Ten minutes prior to milking, cows were administered 1.5 mL of acepromazine maleate (10 mg/mL, i.m.) and 1.0 mL of oxytocin (20 USP units/mL) and oxytocin was given immediately before milking to facilitate milk letdown. Milk was weighed on a digital platform scale and adjusted to a 24 h basis [(milk weight/14) × 24]. A commercial dairy laboratory was utilized for milk quality analysis, which included estimates of milk fat, protein, urea nitrogen, somatic cell count (SCC), lactose, and solids-not-fat (SNF).

**Horn Fly Counts.** Total horn fly populations were recorded on individual animal while in their pasture from 0700 through 0900

hours (h) every 28 days (d) beginning in May and ending in October. Horn flies were treated monthly using Co-Ral® (organophosphate; Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, Kan.), when populations exceeded threshold levels (>200 flies/animal) and after horn fly counts were recorded.

**Blood Serum Hormone Analysis.** Blood samples were collected monthly, beginning in May and ending in October, via jugular venipuncture using vacutainers (Bectin Dickinson, Franklin Lakes, N.J.). Samples were allowed to clot for 24 h at 4 °C, and centrifuged at 2,500 × g for 25 min (minutes) at 4 °C (Marathon 22KBR, Fisher Scientific, Hermle-Labortechnik, Germany). Serum was then harvested and stored at -20 °C pending analysis.

Prolactin concentrations were analyzed in duplicate by double-antibody RIA using primary antisera, purified standard, and iodination preparations supplied by the National Hormone and Peptide Program (Torrance, Calif.).

**Genotyping.** Blood samples were collected via jugular venipuncture using vacutainers (Bectin Dickinson, Franklin Lakes, N.J.) containing EDTA. Samples were centrifuged at 2,500 × g for 25 min at 4 °C (Marathon 22KBR, Fisher Scientific, Hermle-Labortechnik, Germany) to isolate buffy coat. Buffy coat was harvested and stored at -20 °C until genomic DNA isolation using a Qiagen extraction kit (Qiagen Inc. Valencia, Calif.). DNA was diluted to 20 ng/μL prior to sequencing.

Polymerase chain reaction (PCR) primers [forward (5'-AAGTC CCCATAAGCACACTTGG-3') and reverse (5'-CTAACTTTAGGG AGTTCATACTG-3')] were synthesized and supplied by Sigma-Genosys (St. Louis, Mo.). Primers were used to amplify a 500-base segment of the bovine PRL promoter region (position -892 to -1,392; Gen-Bank accession numbers AY337763 and AY641989). A genomic DNA template of 100 ng was added to the amplification reaction (50 μL total volume), which contained 2 μL of each primer and 45 μL of platinum PCR Superimx (Invitrogen, Carlsbad, Calif.). A Peltier thermal cycler (MJ Research, Waltham, Mass.) was used for PCR. The PCR protocol consisted of an initial 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s (second), 55 °C for 1 min, and 68 °C for 1 min. The reaction was completed with 68 °C for 10 min and then held at 8 °C. Amplification products were verified by electrophoresis using 2% agarose gels stained with ethidium

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bromide in 1.0× Tris/Boric Acid/EDTA. Amplicons were purified using the QIAquick PCR purification kit (Qiagen Inc., Valencia, Calif.). Purified PCR products were sequenced at the University of Arkansas DNA Core Lab using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif.).

Sequences were examined using Bioedit Sequence Alignment Editor (Version 7.0.9.0; <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) and compared using the web-based software ClustalW (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>; European Bioinformatics Institute, Cambridge, U.K.). A transversion consisting of cytosine (C) to thymine (T) was identified at position -1,286 in the promoter region of the PRL gene. Three genotypes were observed: homozygous cytosine (CC), homozygous thymine (TT), and heterozygous (CT). Assessment of the sequence chromatograms using the ABI Prism® Sequence Scanner V1.0 (Applied Biosystems, Inc. Foster City, Calif.) and Bioedit allowed for homozygous and heterozygous allele identification.

**Statistical Analysis.** Data were analyzed using PROC MIXED (SAS Institute Inc., Cary, N.C.). The initial linear model for milk traits and cow horn fly count included sire breed (fixed), genotype (fixed), sire breed × genotype (fixed), cow nested in sire breed and genotype (random), month (fixed repeated), month × sire breed (fixed), month × genotype (fixed), month × sire breed × genotype (fixed), and a random residual effect. Models were reduced when the observed significance levels of F tests were greater than 0.25 according to standard procedures for model reduction. Least squares means and standard errors of milk quality and quantity were determined, and mean comparisons were done using *t* statistics, where  $P < 0.10$  denoted a difference.

## Results and Discussion

Distribution of genotypes (C1286T) from the promoter region of the PRL gene among the sire breeds is presented in Table 1. Means of milk production and horn fly counts for the three identified genotypes are reported in Table 2. Statistical analysis of data collected in this study determined PRL genotypes (C1286T) did not have an effect ( $P > 0.25$ ) on milk yield, quality traits or horn fly resistance.

Single-nucleotide polymorphisms within a gene may account for phenotypic variations observed (Sylvänen, 2001). Therefore, the lack of association among genotypes in the promoter region of the PRL gene (C1286T) with milk production traits and horn fly count in this study was unexpected.

The results indicate no observed genotypes (CC, CT, TT) from the promoter region of the PRL gene (C1286T) were associated with milk production or horn fly resistance. Other studies have evaluated the association of SNP in the promoter region of the PRL gene with milk production in dairy cattle, and found SNP (A1043G) to be associated with milk yield (Lü et al., 2010). Cows' homozygous guanine (GG) had increased ( $P < 0.01$ ) milk yield, while cows' homozygous adenine (AA) had increased ( $P < 0.01$ ) milk fat content (Lü et al., 2010). However, research on SNP of the promoter region of the PRL gene in beef cows and their association with milk production traits is lacking. The lack of a significant association between the SNP genotypes (C1286T) and milk production traits and horn fly resistance suggests this polymorphism may not be useful for selecting improved milk production and horn fly resistance in beef cows.

## Implications

Results suggest the single nucleotide polymorphism of the prolactin gene examined in this study may not be useful when selecting beef cows for milk production traits and horn fly resistance. Further studies of the association of genetic polymorphisms with these traits is needed to identify useful markers for the development of new selection and management strategies.

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**Table 1. Distribution of genotypes among sire breeds.**

Sire Breed	Genotype		
	CC	CT	TT
Bonsmara	1	3	3
Brangus	5	8	0
Charolais	3	2	3
Gelbvieh	0	4	1
Hereford	4	4	4
Romosinuano	3	2	3

CC = Homozygous cytosine; TT = Homozygous thymine; CT = Heterozygous cytosine/thymine.

**Table 2. Milk production means for genotypes with pooled standard deviation.**

Variable	Genotype			SD
	CC	CT	TT	
Horn Fly Count	351.00	322.00	338.00	303.00
Milk Yield	7.21	7.56	6.94	2.53
Milk Fat	3.50	3.41	3.26	1.04
Milk Protein	3.30	3.17	3.14	0.45
Milk Lactose	4.86	4.74	4.89	0.49
Milk Urea Nitrogen	11.97	11.00	10.90	4.64
Somatic Cell Count	277.34	173.56	115.57	529.84
Solids-Not-Fat	9.11	8.83	8.96	0.85
Prolactin	35.87	82.29	61.09	79.79

CC = Homozygous cytosine; TT = Homozygous thymine; CT = Heterozygous cytosine/thymine. Serum prolactin (ng/mL); Milk yield (kg/d); Milk fat, milk protein, milk lactose, and solids-not-fat (%); Milk urea nitrogen (mg/dL); Somatic cell count (count x 10); ( $P > 0.25$ ), SD = pooled standard deviation.

# Effects of ergot alkaloids on bovine sperm motility

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## Story in Brief

Toxic tall fescue grass has been associated with reduced reproductive rates in cattle. This study was conducted to determine the direct effects of the ergot alkaloids [ergonovine (EN), ergotamine (ET), and dihydroergotamine (DHET)] on motility of bovine spermatozoa. Spermatozoa were collected from mature Angus (n = 2) and Balancer (n = 4) bulls, washed once, and resuspended in modified sperm medium (mSPTL). The experimental design was a randomized complete block, with bull serving as the block. Treatments were structured as a 3 × 5 factorial with three alkaloids (EN, ET, DHET) and five concentrations of each drug (0, 33, 66, 100, and 200 μM). Spermatozoa (25 × 10<sup>6</sup>) were incubated in 1 mL of mSPTL with treatment at 39 °C. Sperm motility characteristics were evaluated at 0, 3, and 6 h using CASA (Hamilton Thorne IVOS, Beverly, Mass.). Initial sperm motility was (69 + 1.7%) and declined to (35 + 2.6%) at 6 h. Percent motile spermatozoa was affected (P = 0.015) by a three way interaction between time, concentration, and alkaloid. Sperm motility decreased (P < 0.01) over time and with increased concentrations of alkaloids with the exception of EN. The number of static spermatozoa also was affected (P < 0.01) by a three way interaction and increased as ET and DHET concentrations increased. Percentages of progressively motile and rapidly motile spermatozoa decreased (P < 0.01) in a two way interaction between alkaloid and concentration. Overall sperm motility was decreased by ET and DHET; furthermore, the qualities of motility were decreased by those alkaloids. Ergot alkaloids commonly found in toxic tall fescue are detrimental to bovine spermatozoa.

## Introduction

Cattle consuming toxic tall fescue (E+) may suffer from numerous detrimental effects, resulting in significant economic losses for cattle producers annually. Three-fourths of all tall fescue pastures in the United States are infested with endophyte at a level of at least 60%. Ergot alkaloids located within the endophytic fungus have been linked to depressed reproductive performance.

Bulls consuming E+ and ergot alkaloid may suffer from altered scrotal temperatures, decreased scrotal circumference (Jones et al., 2004), declines in sperm motility (Looper et al., 2009), and reduced fertilizing capabilities (Schuenemann et al., 2005). Collectively, results from those in vivo studies suggest that toxic fescue can alter sperm function and fertilizing capabilities; however, they do not illustrate what specific effects ergot alkaloids may have directly on spermatozoa. Wang et al. (2009) showed that certain ergot alkaloids use specific signaling pathways to interact with spermatozoa. Our objective was to investigate the direct effects ergot alkaloids on bovine specific sperm motility characteristics by using computer assisted sperm analysis.

## Materials and Methods

**Semen Collection and Preparation.** Semen was collected from mature bulls (n = 6) via electro-ejaculation (Electro-ejac IV) at 0700 h and placed in a 15 mL conical centrifuge tube. Ejaculates were transported to the lab in a 39 °C water bath where spermatozoa were centrifuged at 750 × g for 10 min. Seminal plasma was removed, and spermatozoa were washed once and re-suspended in modified sperm TALP (mSPTL). The mSPTL was prepared prior to collection and consisted of: NaCl (49.5 mM), KCL (1.5 mM), NaH<sub>2</sub>PO<sub>4</sub> (0.17 mM), CaCL<sub>2</sub>·2H<sub>2</sub>O (0.10 mM), MgCL<sub>2</sub>·2H<sub>2</sub>O (0.055 mM), 5.25% NaHCO<sub>3</sub> (0.16 ml), HEPES (10 mM), Na-pyruvate

(1 mM), 60% Na-lactate syrup (21.6 mM), gentamicin (0.05 mg), EGTA (2 mM), and PVA (0.05 mg) with pH adjusted to 7.4 and an osmolarity of ~300 mOsm. Spermatozoa were diluted 25:1, counted using an integrated visual optical system (IVOS; Hamilton-Thorne Biosciences, Beverly, Mass.), and placed in experimental treatments (25 × 10<sup>6</sup> sperm/ml).

**Preparation of Alkaloid Treatments.** All alkaloids were prepared directly prior to incubation with spermatozoa. Methanol (100%) was used as the solvent to prepare each alkaloid [ergonovine (EN), ergotamine (ET), dihydroergotamine (DHET)]. Stock solutions were aliquoted at experimental concentrations into the wells of sterile flat-bottom 24-well tissue culture plates and methanol was allowed go evaporate. Alkaloids were then re-suspended in mSPTL.

**Experimental Design.** Experimental design was a randomized complete block, with bull serving as the block. Treatments were structured as a 3 × 5 factorial with three alkaloids (EN, ET, DHET) and five concentrations of each alkaloid (0, 33, 66, 100, 200 μM). Spermatozoa (25 × 10<sup>6</sup>) were incubated in 1 mL of mSPTL with each treatment at 39 °C in an atmosphere of humidified air. Sperm motility characteristics were evaluated at 0, 3, and 6 h of incubation. Spermatozoa were evaluated by placing them on a warm slide and assessed using an IVOS and utilizing Animal Motility Software, version 12.1.

**Statistical Analysis.** Sperm motility characteristics were analyzed using mixed model procedure (SAS Institute, Inc, Cary N.C.). Bull served as the block, experimental unit was the concentration within alkaloid, and time was repeated measure. If F-test were significant (P < 0.05), means were separated using multiple t-tests.

## Results and Discussion

Sperm motility was inhibited by a three-way interaction between hour (time), alkaloid, and concentration. Both ET and DHET re-

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duced sperm motility in a concentration and time dependent manner ( $P < 0.05$ ). When compared to control spermatozoa (0  $\mu\text{M}$  alkaloid), spermatozoa exposed to ET ( $\geq 33 \mu\text{M}$ ) for 3 h and ( $\geq 66 \mu\text{M}$ ) for 6 h were less motile ( $P < 0.05$ ; Fig. 1). Similar affects were observed for DHET. Concentrations of 66  $\mu\text{M}$  and above reduced ( $P < 0.05$ ) sperm motility (Fig. 2). Ergonovine had minimal effects on sperm motility until the 6 h observation at 200  $\mu\text{M}$  ( $P < 0.05$ ). Similarly, static spermatozoa also were affected by a three-way interaction between alkaloid, concentration, and time. As ET and DHET concentrations increased, the number of static spermatozoa increased. For progressive and rapid spermatozoa, a two-way interaction was observed. Both ET and DHET reduced ( $P < 0.05$ ) the percentage of progressive (Fig. 3) and rapid (Fig. 4) spermatozoa when concentrations reached 66  $\mu\text{M}$  and greater.

There has been conflicting data published showing the effects of toxic agents found in E+ on male gametes, but our results demonstrate that ergot alkaloids can directly affect bovine sperm motility. More specifically, ET and DHET reduced motile, progressive, rapid, and static spermatozoa and altered multiple other sperm characteristics associated with sperm viability. These data provide a possible explanation for decreased conception rates and reproductive performance amongst cattle grazing toxic tall fescue.

The altered sperm parameters observed in our study were similar to Wang et al. (2009); however, their method of evaluating sperm motility utilized subjective measures. According to Farrell et al. (1998), the repeatability and consistency within each sperm evaluation is likely to be more accurate using CASA rather than subjective measures. In fact, Farrell's group reported a repeatability of 0.99 when using CASA. This could also provide a valid explanation for the contrasting results encountered with many other trials. For example, with the use of visual optics, Schuenemann et al. (2005) documented that sperm motility and morphology was not affected when bulls were supplemented with ET in their diet.

The use of CASA allowed us to evaluate both quality and quantity of sperm motility. Although sperm movement is important, it is not the only criterion necessary for a sperm to fertilize an oocyte. The ability of the sperm to progress forward into the reproductive tract in an efficient manner is critical to achieve conception. Results, in this report, showed declines in overall motility and reductions in progressive and rapid spermatozoa. These results confirm our earlier work where the average velocity of the smoothed sperm path as well as the average velocity measured over the actual point to point track became slower with elevated temperatures (Looper et al., 2009). We also demonstrated that the percentage of static spermatozoa increased due to the effects of ET and DHET, which exasperate the intracellular energy of the sperm, and thereby accelerate the pace at which sperm undergo apoptosis. We observed morphological changes in the size and shape of the sperm head as exposure time and alkaloid concentration increased.

Eliminating subjective measures for sperm evaluation is not the only methodology that differs in our study as compared to others. Multiple trials have been performed showing that ergot alkaloids can indirectly affect sperm function. The previously mentioned alterations in scrotal temperature and scrotal circumference along with changes in prolactin concentrations are physiological changes that occur after ingestion of E+ (Jones et al., 2004), and these changes may be partially responsible for reducing sperm viability under normal physiological conditions. It is known that thermal regulation of the testis and prolactin levels are both important factors that can regulate the development of sperm.

In two of the more current whole animal studies, both Looper et al. (2009) and Jones et al. (2004) observed a small decline in sperm

motility. Although it is important to understand how cattle react to toxic agents under normal grazing conditions, it is important to note that breed type and exposure period may have affected their results. Looper's trial utilized Brahman-influenced bulls which are known for their heat tolerance; therefore, it may be that Brahman-influenced bulls withstand the toxic effects of E+ better than other breed types. Even though sperm motility was not greatly affected over the entire length of the study, Jones' article did state that motility decreased during the final two weeks of the 60 d trial. Perhaps bulls with a longer exposure period may begin to respond differently. It is also known that elevated environmental temperatures can magnify the effects of toxic fescue. Ultimately, there are many factors that could possibly influence whole animal trials such as breed type, exposure period, toxin concentrations, temperature, and body weight. By taking an in vitro approach to this study we were able to determine if ergot alkaloids directly interact with spermatozoa. As we try to discover different alternatives to combat fescue toxicosis, it is important that we understand all mechanisms and specific alkaloids that possibly reduce the animals reproductive capabilities.

It is still unknown exactly what mechanisms ergot alkaloids use to inhibit sperm motility. The chemical structures of the three alkaloids used in this study could possibly explain the observation differences amongst the alkaloids. Ergonovine, the smallest structure of the three, is a simple lysergic acid amide that doesn't contain a peptide group. Ergonovine decreased sperm motility when exposure occurred during cryopreservation, and another demonstrated that EN increased rate of sperm transport when placed in the vagina of ewes. Even though we observed a reduction in sperm motility when exposed to large amounts of EN (200  $\mu\text{M}$ ), overall, the data from this current study showed EN to have a minimal effect on bovine sperm motility. Both ET and DHET are classified as ergopeptines. Inhibitory effects of DHET were slightly less intense than ET, which was not expected since DHET was originally synthesized to be a more stable version of ET for use in human pharmacology. Ergot alkaloids are lipid soluble and presumably can permeate sperm membranes. Sperm motility is dependent on many cellular functions including cAMP and calcium concentrations. It is plausible that ergot alkaloids can directly affect sperm motility by altering cAMP and calcium levels within the germ cell. Fertilizing capacity and motility also may be compromised by ergot alkaloid interaction with plasma membrane receptors on spermatozoa (Wang et al., 2009).

It is unlikely that producers will eradicate all of their E+ stands due to its persistence and agronomic benefits, so establishing a method to help overcome the negative consequences toxic fescue has on reproductive performance seems to be the more feasible option. This study provides a better understanding of the effects ergot alkaloids have on male reproduction. Unfortunately, ergot alkaloid concentrations are not known under normal physiological conditions, but with the knowledge that these toxic agents can directly hinder sperm motility, we can address the mechanisms used to inhibit sperm function.

## Implications

Understanding the mechanisms by which ergot alkaloids and toxic tall fescue reduce male reproduction may lead to management tools that will result in increased overall livestock reproductive success.

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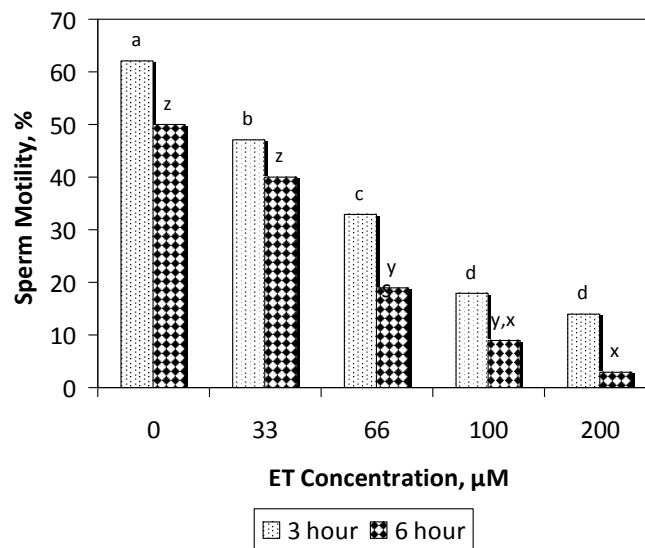
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**Fig. 1.** Effects of ergotamine tartrate (ET) concentration and incubation time on bovine sperm motility. Spermatozoa ( $25 \times 10^6$  sperm/ml) were incubated with ET at various concentrations (0-200  $\mu\text{M}$ ) in modified sperm TALP (mSPTL). Sperm motility characteristics were evaluated at 0, 3, and 6 h. Initial motility was 68% and SE = 4.1. Superscripts a,b,c,d are designated to 3 h columns and superscripts z,y,x are designated to 6 h columns. Values without a common superscript within evaluation time differ ( $P < 0.05$ ).

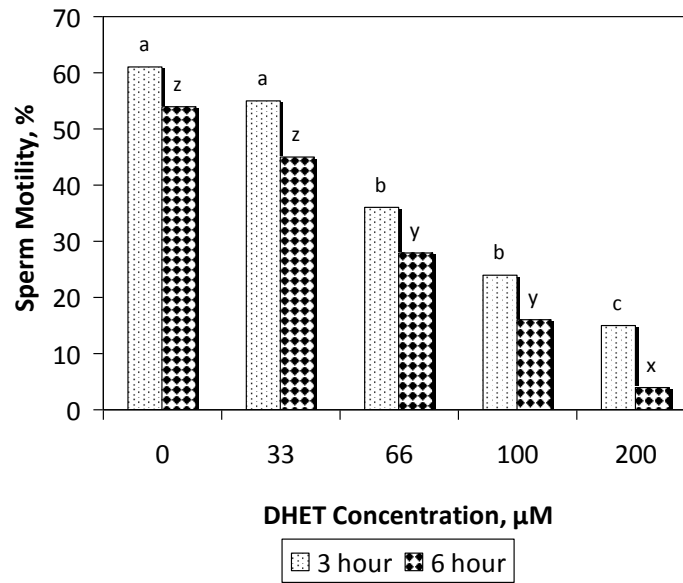


Fig. 2. Effects of dihydroergatamine (DHET) concentration and incubation time on bovine sperm motility. Spermatozoa ( $25 \times 10^6 \text{ ml}^{-1}$ ) were incubated with DHET at various concentrations (0-200  $\mu\text{M}$ ) in modified sperm TALP (mSPTL). Sperm motility characteristics were evaluated at 0, 3, and 6 h. Initial sperm motility was 67% and SE = 4.1. Superscripts a,b,c are designated to 3 h columns and superscripts z,y,x are designated to 6 h columns. Values without a common superscript within an evaluation time differ ( $P < 0.05$ ).

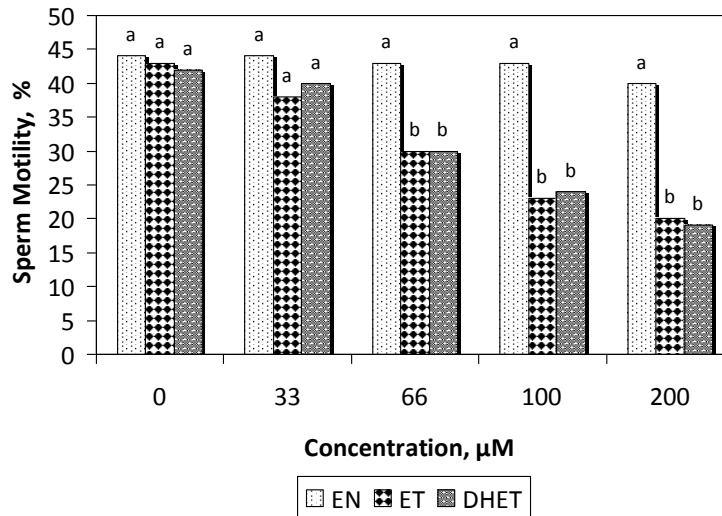
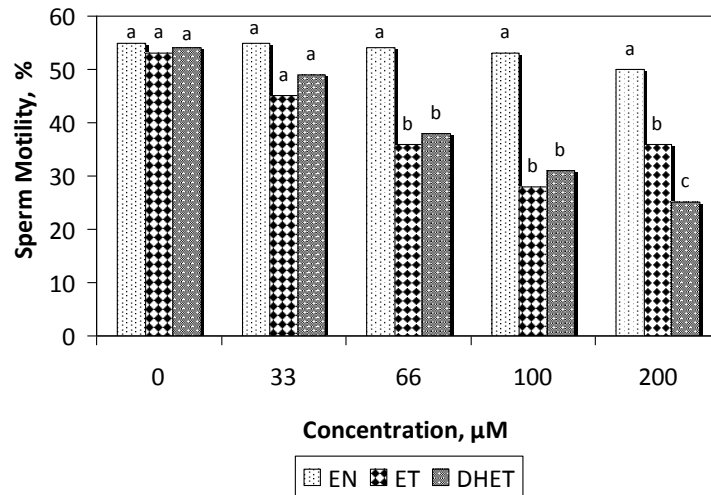


Fig. 3. Effects of alkaloid and concentration interaction on progressive sperm motility. Spermatozoa ( $25 \times 10^6 \text{ ml}^{-1}$ ) were incubated with alkaloid [ergonovine (EN), ergotamine tartrate (ET), dihydroergotamine (DHET)] at various concentrations (0-200  $\mu\text{M}$ ) in modified sperm TALP (mSPTL). SE = 2.1. Values without a common superscript differ ( $P < 0.05$ ).





**Fig. 4.** Effects of alkaloid and concentration interaction on rapid sperm motility. Spermatozoa ( $25 \times 10^6 \text{ ml}^{-1}$ ) were incubated with alkaloid [ergonovine (EN), ergotamine (ET), dihydroergotamine (DHET)] at various concentrations (0-200  $\mu\text{M}$ ) in modified sperm TALP (mSPTL). SE = 3.5. Values without a common superscript differ ( $P < 0.05$ ).

# Clover emergence and biomass production in wooded areas

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## Story in Brief

Landowners in Arkansas who manage considerable amounts of wooded areas are interested in improving wildlife habitat. In this study, arrowleaf (*Trifolium vesiculosum* Savi) and white clover (*Trifolium repens* L.) in pine (*Pinus* spp.) tree alleys of varying width (12, 16, 24, and 32 feet) were planted to test for clover establishment success and dry matter production. Pine alleyways were cleared of woody vegetation, disked at a shallow depth, fertilized and limed, and planted with a broadcast seeder. Preliminary results suggested in March that dry matter production of both clovers in the open control area (no shade) was about twice the production in the 24- and 32-foot alleyways. By June, dry matter production of arrowleaf clover was about 4,000 lbs/acre (70% of the open area), and that of white clover 3,000 lbs/acre (65% of the open area). There was no difference in dry matter production between the unfenced plot areas and exclusion cages, suggesting little grazing pressure by deer during the first half of 2012. White clover disappeared entirely from the open area during the severe summer drought of 2012 but persisted under shade in some areas, although at a very low canopy height (below 1.5 inches), which made forage mass collection unfeasible for the rest of the growing season. Our results suggested that both arrowleaf and white clovers can be successfully established. Weed pressure in white clover plots may heavily affect persistence of beyond the first year of growth. Arrowleaf clover is highly competitive, but needs to be reestablished every year.

## Introduction

Highly digestible forages have traditionally been used by hunters to improve habitat with the purpose of attracting deer and other game. Research data on this issue is scarce, partly because of difficulties in controlling environmental factors during data collection and controversy surrounding the validity of food plot research (Moorman et al., 2006). In this experiment, arrowleaf (*Trifolium vesiculosum* Savi) and white clover (*Trifolium repens* L.) were planted in pine (*Pinus* spp.) tree alleys of varying width (12, 16, 24, and 32 feet) to determine the establishment success and dry matter (DM) production of these clovers.

## Materials and Methods

The research was conducted at the USDA-ARS Small Farms Research Unit in Booneville, Ark. Fifteen-year old pine tree alleys of varying width (12, 16, 24, and 32 feet) were selected for this study. An open area was included as control. In the fall of 2011, 3 alleys selected for each treatment were cleared of woody vegetation by hand and raked with a commercial hay rake (Vermeer, Pella, Iowa) to remove pine needles. Each plot received the equivalent of 4,000 lbs lime, 80 lbs phosphate, and 80 lbs of potash per acre. To control weeds and to prepare a smooth seedbed, plots were then disked at a maximum depth of 5 inches to avoid damage to pine tree roots. Control plots were prepared in a similar fashion. Legumes were planted at rates of 20 lbs/acre for variety-not-stated (VNS) arrowleaf and 10 lbs/acre 'Ivory 2' white clover, using a Brillion broadcast seeder (Brillion Co., Brillion, Wis.) on October 14, 2011. This machine is equipped with packing rolls in the front and back of the seed box so that soil is sufficiently compacted during the planting process. Arrowleaf clover, an annual clover, was replanted on October 17, 2012. White clover, a perennial species, was not replanted. Exclusion cages measuring 16 by 16 feet were set up in February of 2012. At the same time, light meters and soil

temperature probes (Spectrum, Inc., Aurora, Ill.) were installed in each treatment plot.

Seedling counts were performed 4 weeks after establishment using a wire grid structure placed in each plot. Both species were subjected to a similar species count in fall of 2012. For arrowleaf clover, emerged seedlings were counted; and for white clover, the number of remaining plants from the previous season was recorded using the same method.

Dry matter production was assessed by clipping quadrats measuring 100 square inches, leaving a 1.5-inch residue height, with a gas-powered hedge trimmer. Plots were clipped monthly from March to September, collecting one sample outside the cage and one inside the cage, in each experimental unit. Samples were placed in paper bags and dried at 125 °F in a forced-air oven until no further weight loss was detected for determining DM yield.

Data was analyzed as a completely randomized design with a factorial arrangement of treatments. The MIXED procedure of SAS was used to determine possible treatment effects and interactions between species and alley widths and was calculated separately for each time of sampling. Least-significant differences (LSD) were calculated for the interaction term. Differences of means were considered significant at  $P < 0.05$ .

## Results and Discussion

Significant ( $P < 0.05$ ) effects of alley widths, species, and their interactions on seedling counts were observed for both dates of sampling (Fig. 1). The relatively high count of seedlings for both species in the 12-foot alley in November of 2011 was possibly caused by less weed competition or higher moisture retention compared with other treatments, offsetting reduced light penetration. Seedling counts for arrowleaf clover in November of 2012 appeared to be similar to the previous year. White clover disappeared almost entirely during 2012 due to severe drought. The single bar for the 16-foot treatment (November 2012; Fig. 1)

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was associated with additional 16-foot alleys required to reestablish this species along with arrowleaf clover because the originally used alleys became flooded that killed all clover plants. By January 2013, some white clover plants had reemerged (data not shown). Since there was no visible evidence of white clover plants the previous fall (except for the reestablished 16-foot alley treatment), those few plants presumably emerged from seeds that were shed during 2012 or regrowth from dormant stolons. Although white clover is a perennial plant, flowering and seed setting occur continuously during the growing season and are in fact necessary for the long-term survival of this legume. In the open area, no seedlings were detected after January 2013 (data not shown) because the drought during 2012 had a much more severe effect there than in the shaded alleys where moisture and soil temperature remained more favorable for clover growth.

Forage mass production in the open plot area ranged from less than 1,000 lbs/acre to more than 5,000 lbs/acre in 2012 depending on species and time of sampling (Fig. 2). As expected, DM production was greater in the open area compared with the alleys (open plot area), but differences appeared to be less pronounced in June. There were no apparent differences in DM production between the caged and the open plot area. No forage mass could be obtained for white clover beyond June due to drought conditions, although this species did not cease growth entirely. Not surprisingly, white clover plots became increasingly infested with weeds which may affect the second-year persistence of this species. Arrowleaf clover concluded its life cycle at the end of June and was not sampled further either.

The biomass from this clover decayed over the course of the summer and was finally incorporated into the soil prior to reestablishment in October of the same year.

The small amount of forage mass collected from the 16-foot alley is the result of repeated flooding after rain events that impeded biomass growth. As a result, plots were reestablished in the fall of 2012 in newly selected 16-foot alleyways.

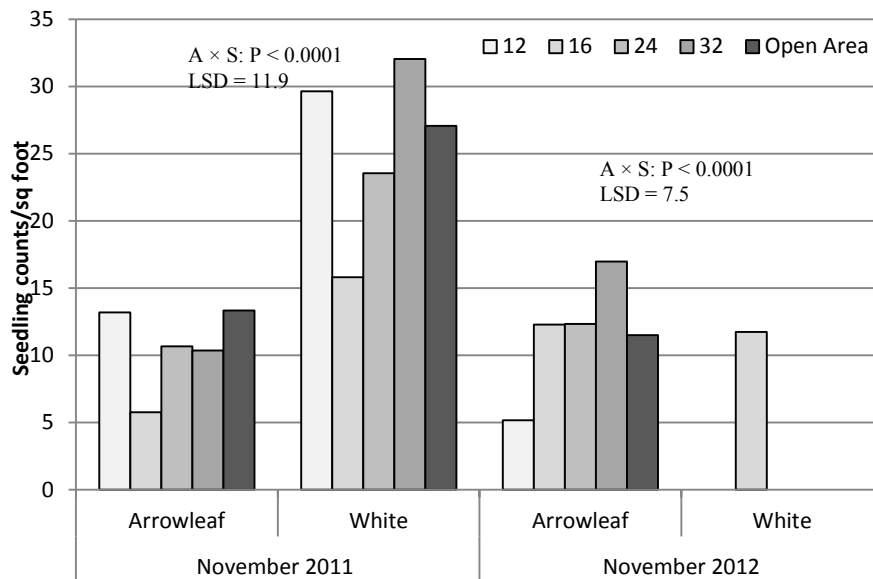
Data for light penetration are currently being analyzed. Preliminary results indicated that light penetration in the pine alleyways was as low as 25% (12-foot alley) compared with that measured in the open area.

## Implications

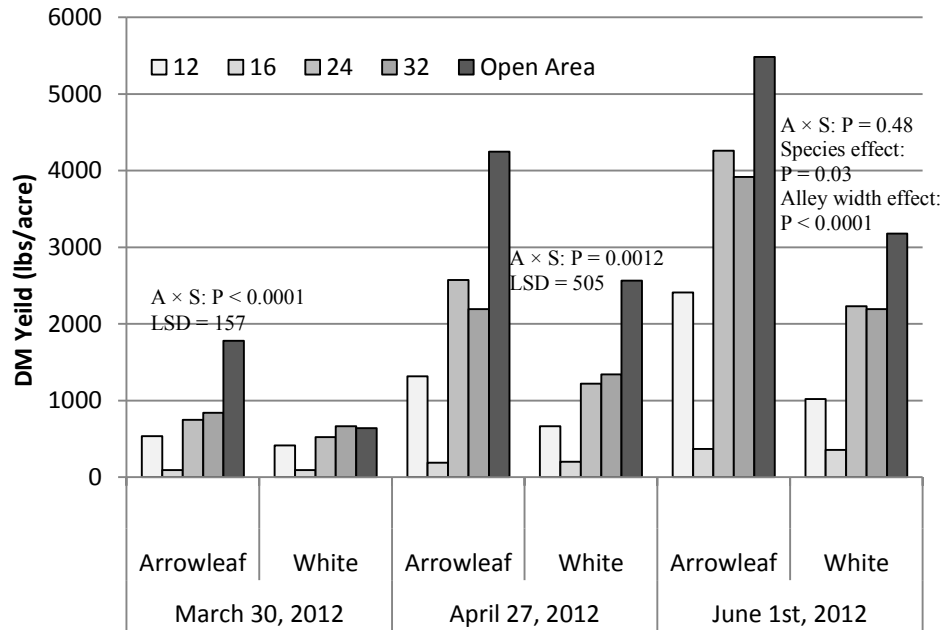
Arrowleaf and white clover can be established in shaded wooded areas. Arrowleaf clover is very competitive but has to be reestablished every year. White clover productivity seems to be greatly reduced long-term, due to increasing weed pressure. Research regarding planting times should be conducted to synchronize nutritional needs of wildlife with growth in food plots.

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**Fig. 1. Seedling counts for arrowleaf and white clovers on two different dates for each alley treatment (12, 16, 24, and 32 feet plus open area). Each date was analyzed separately,  $P$ -values for the interaction (alley width  $\times$  species =  $A \times S$ ) and LSD ( $t_{\alpha/2} = 0.025$ ) for the interaction term within a single date are displayed. The single bar in Nov 2012 for white clover resulted from replanting the 16-foot alley width after clover failure due to repeated flooding during 2012. There were no white clover plants left in any other treatment due to a severe drought in 2012.**



**Fig. 2. Dry matter (DM) yields for arrowleaf and white clovers on three different dates for each alley treatment (12, 16, 24, and 32 feet plus open area). Each date was analyzed separately, *P*-values for the interaction (alley width × species = A × S) including LSD ( $t_{\alpha/2} = 0.025$ ) for the interaction term (except for the June date with an insignificant interaction) and other effects are displayed. Data is from the unfenced plot areas only; data from within cages is not shown. The low forage mass in the 16-foot alleys were caused by repeated flooding of plots.**

# Effect of hormonal growth implant administration timing on health, performance, and immunity of beef stocker cattle

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## Story in Brief

The study objective was to determine the effects of different time of administration (study day 0, 14 or 28) of a growth implant containing 200 mg progesterone and 20 mg estradiol benzoate (Synovex S, Zoetis, Madison, N.J.) on health, growth and immune alteration in newly received beef calves utilized in a receiving/grazing stocker system. We hypothesized that efficacy (growth response) of an exogenous growth implant is reduced when administered to calves during stress-induced immune dysfunction; whereas, health and immunity are altered by the time of administration. However, our results indicate that although body weight gains were not statistically different during the receiving period, overall performance during the entire stocker ownership period was increased similarly for implanted calves vs. negative control, regardless of time of implantation. Although, gains on pasture in 2 blocks of this study were increased with delayed implanting, there was no benefit to delayed implantation on overall (receiving plus grazing) performance. Administration of a hormonal growth implant did not affect clinical morbidity or bovine viral diarrhoea virus titer concentrations. Therefore, under conditions of this study, the time of growth implant administration did not affect growth implant efficacy, health, or vaccine response in beef stocker calves. Our overall observations suggest that there is not a benefit to delaying growth implantation in newly received beef calves.

## Introduction

Cattle experiencing stress-induced physiological alterations and immune dysfunction are commonly received into stocker or backgrounding programs because additive factors such as weaning, commingling, handling, and transportation during the cattle marketing process. The hypothesis that stress may reduce growth implant efficacy has not been adequately explored. Implanting with exogenous growth promoting hormones during stressful periods may impact the immune response because actions of the anabolic hormone could modify metabolism to enhance growth factors in exchange for energy required for immunity when bovine respiratory disease (BRD) is most prevalent. Therefore, on-arrival administration of a growth implant could potentially result in decreased efficacy of the growth implant or increased morbidity due to interactions of metabolic and immunologic host factors and the physiologic actions of the anabolic hormone released by the growth implant during stress-induced immune dysfunction.

Research is needed to determine the optimum timing of administration of growth implants for high-risk, newly received beef calves. Therefore, effects of on-arrival (d 0) versus delayed (d 14 or 28) timing of implantation using a conventional implant product (Synovex S) on animal health, performance, bovine viral diarrhoea virus (BVDV) titer (humoral immune response), and total and differential leukocyte counts (immunomodulation) were evaluated during a receiving period and subsequent grazing period.

## Materials and Methods

Experimental procedures followed standard protocol used at the University of Arkansas Livestock and Forestry Research Station located near Batesville, Ark. Animal methods were approved by the University of Arkansas Animal Care and Use Committee.

Experimental treatments consisted of: 1) negative control (no growth implant administered), 2) Synovex S (Zoetis, Madison, N.J.) growth implant administered on-arrival (d 0), 3) Synovex S administered on d 14, and 4) Synovex S administered on d 28. Treatments were evaluated in a randomized complete block design with inferences made on growth performance, BRD morbidity rate, percentage chronic (nonresponsive), mortality, and days to first antimicrobial treatment. Furthermore, 5 randomly selected animals from each treatment pen were bled via jugular venipuncture and serum was pooled within pen to evaluate vaccine response (BVDV type 1a titers) on d 0, 14, 28, and 42. A complete blood count via automated hemocytometer (Cell-Dyn, Abbott Laboratories) was used to determine immune alteration (total and differential peripheral blood leukocyte concentrations, hemoglobin, hematocrit, platelets) also on d 0, 14, 28, and 42. Upon completion of the receiving phase of the trial, calves were commingled and allowed to graze small grain pasture for an additional 78 d.

Male beef cattle ( $n = 399$ ,  $488 \pm 58.7$  lb) were received at the University of Arkansas Livestock and Forestry Research Station near Batesville on 3 dates: September 12, 2011 (Block 1,  $n = 106$  calves); October 31, 2011 (Block 2,  $n = 100$  calves); and January 9, 2012 (Block 3,  $n = 193$  calves). After arrival at the unit (d -1), calves were weighed individually, tagged in the ear with an individual identification tag, ear notched and tested for persistently infected (PI)-BVDV status at a commercial laboratory (CattleStats LLC, Oklahoma City, Okla.), and castrate status was determined. No experimental calves tested positive for PI-BVDV. Calves were stratified by d -1 body weight and castrate status (bull or steer), then assigned randomly to pen (8 pens for Blocks 1 and 2, 12 pens for Block 3; 12 to 17 calves/pen). Treatments were assigned randomly to pen. Pen was considered the experimental unit, and treatments were replicated 2 times during Blocks 1 and 2, and 3 times in Block 3 resulting in a total of 7 pen replicates for each treatment in the

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study. The following day (d 0) calves were weighed, bled, vaccinated with a pentavalent (bovine herpesvirus-1, BVDV type 1a and 2a, bovine respiratory syncytial virus, parainfluenza-3 virus) modified live virus vaccine (Bovi-Shield GOLD 5, Zoetis), a multivalent clostridial/*Manheimia haemolytica* bacterin-toxoid approved for single-dose efficacy (One Shot Ultra 7, Zoetis), and a concentrated tetanus toxoid (Colorado Serum Company, Denver, Colo.). Calves were dewormed (Dectomax, Zoetis), and castrated by California banding method if applicable (InoSol Co. LLC, El Centro, Calif.). Also on d 0, calves assigned to the on-arrival implant treatment received a growth promoting hormonal implant supplying 200 mg progesterone and 20 mg estradiol benzoate (Synovex S, Zoetis) administered subcutaneous in the caudal aspect of the right ear. On d 14 or 28, calves assigned to the appropriate implant treatment were administered the Synovex S growth implant.

After initial processing, calves were fed an identical receiving supplement at rates increasing up to a maximum of 4 lb/d with ad libitum access to bermudagrass hay (10% crude protein; 57% total digestible nutrients). Bunks were checked each morning at approximately 8:00 a.m., if feedbunks were clean the amount of supplement offered was increased by 0.5 lb/calf until the 4 lb/d rate was reached. After the quantity of feed to be provided to each pen was determined, that amount was weighed and hand fed each morning at approximately 8:30 a.m.

Block 1 steers were weighed on 2 consecutive days at the initiation of the experiment (d -1 and 0), d 14, 28, 42, 63, 64, 91 and on consecutive days at the end of the grazing period (d 119 and 120). Although the pre-determined end of the receiving period was d 42, the pastures at the site were not adequately developed for calf turnout in Block 1. Therefore, calves in Block 1 were placed back in their assigned receiving pens until forage availability was sufficient for grazing turnout on d 64.

Block 2 calves were weighed on 2 consecutive days at the initiation of the experiment (d -1 and 0), d 14, 28, 42, 43, 64, 91 and on consecutive days at the end of the grazing period (d 119 and 120). On d 43, 91 of the 100 steers received in Block 2 were shipped to the University of Arkansas Southwest Research and Extension Center, near Hope, Ark. (215 miles, 4-hr transit time) for grazing on cool-season annual pastures. Five steers considered to be chronically ill with BRD were not shipped for grazing, and 4 steers did not survive the receiving period.

Block 3 calves were weighed on 2 consecutive days at the initiation of the experiment (d -1 and 0), d 14, 28, 42, 43, 76, and on consecutive days at the end of the grazing period (d 124 and 125).

All calves were observed daily for signs of BRD throughout the receiving and grazing periods. Signs considered indicative of BRD were nasal or ocular discharge, heavy breathing, or depression. If  $\geq 2$  signs existed, calves were brought to the chute and rectal temperature was recorded. If rectal temperature was  $\geq 104$  °F, calves were treated according to a predetermined antimicrobial protocol consisting of initial treatment with tulathromycin (Draxxin, Zoetis); secondary treatment with florfenicol (Nuflor, Merck Animal Health, Summit, N.J.); and tertiary treatment with ceftiofur hydrochloride (Excenel RTU, Zoetis). The post-treatment interval for tulathromycin and florfenicol was 8 and 2 d, respectively.

At the end of each 42-d receiving period (except as described for Block 1), all calves were commingled and assigned to small grain pastures by allocating each experimental treatment equally to pastures. Block 1 steers were allocated to pastures (52 acres of wheat pasture in dedicated crop fields and 44 acres of wheat interseeded into warm-season grass based pastures) at an average stocking rate of 1.06 steers/acre. Calves in Block 1 were allowed to graze wheat

pasture for an additional 56-d grazing period (120-d total).

For Block 2, 91 steers were shipped on December 13, 2011 to the University of Arkansas Southwest Research and Extension Center (SWREC), near Hope. Pastures consisted of bermudagrass that were interseeded to a blend of small grain and annual ryegrass in mid-September. One 30-acre pasture was planted to wheat and ryegrass, a total of 48 acres (in 4 separate pastures) was planted to oats and ryegrass, and 42 total acres (in 6 pastures) was planted to rye and ryegrass. Stocking rate decisions were made independently for each pasture allocating an average forage allowance of 2.9 lb of forage dry matter/lb of body weight, but the average stocking rate across pastures was 1.3 acres/steer.

For Block 3, steers were similarly assigned to the same wheat pastures used for Block 1 starting February 22, 2012. Steers were stocked on pastures at 2 calves/acre and remained on pasture until May 15, 2012 (d 125).

Animal performance data during the receiving period were analyzed with pen as the experimental unit. Steers were commingled across treatments and pens during the grazing period; therefore, performance data recorded during this time was analyzed using individual animal as the experimental unit. A randomized complete block design was employed to analyze the fall and winter data (Block 1 and 2), and a completely randomized design was used in the analysis of the spring data (Block 3). Computations were made with the mixed models procedure of SAS (SAS Inst. Inc., Cary, N.C.). Because the post-receiving body weight data collection events in Block 3 did not correspond with the days post implantation in Blocks 1 and 2, post-receiving performance data from this block was treated as a separate experiment, and analyzed as a completely randomized design. Animal health data were analyzed for the fall and spring periods separately as binomial data using the GLIMMIX procedure of SAS. Hematological parameters and BVDV type 1a titer data were evaluated using the MIXED procedure of SAS with repeated measures.

## Results and Discussion

*Block 1 and 2.* Performance of steers during fall (Block 1) and winter (Block 2) receiving and grazing periods are presented in Table 1. There were no statistical differences ( $P \geq 0.16$ ) in steer body weight or average daily gain (ADG) during the receiving period for Blocks 1 and 2. During the first 21-d of the grazing period (d 42 to 63), steer gains were lower than what would be expected based on forage quality. This phenomenon is common when steers are removed from a dry lot consuming primarily hay-based diets and placed on high quality cool-season annual pastures. Ruminal adaptation to the rapid diet change may take several weeks, and lower than expected performance typically occurs during this transition, as was observed for the current study. During the first 21 d of the grazing period (d 42 to 63), steers that were implanted later in the receiving period (Implant 14 and Implant 28) gained weight faster ( $P \leq 0.01$ ) than control, while ADG of Implant 0 was intermediate. From d 63 to 91, implanted steers gained weight 0.7 lb/d more rapidly ( $P < 0.01$ ) than non-implanted controls. Later in the grazing period from d 91 to 120, steers implanted on d 28 gained more rapidly ( $P \leq 0.01$ ) than steers that were not implanted or were implanted on d 0 or 14, which did not differ ( $P \geq 0.12$ ), indicating that the growth response from implants administered early in the receiving period had decreased at this time, whereas implants administered later (d 28) in the receiving period remained active during the later stage of grazing.

Over the entire fall and winter grazing periods, ADG of controls was 0.44 lb/d less ( $P < 0.01$ ) than calves that received a growth

implant. The ADG of steers implanted on d 0 was 0.16 lb/d less ( $P = 0.05$ ) than those implanted on d 28 and gain of those implanted on d 14 tended ( $P = 0.11$ ) to be 0.13 lb/d less than those implanted on d 28. Overall ADG (receiving plus grazing gain) was 0.25 lb/d greater ( $P < 0.01$ ) for steers across all implant timing treatments compared with control; however, there were no differences ( $P \geq 0.36$ ) in ADG observed for implant timing regimens.

There were no differences ( $P \geq 0.30$ ) in BRD morbidity or the number of days to first antibiotic treatment. Morbidity in the fall was exceptionally high (94% morbidity for all calves). The average day of first antibiotic treatment was 1.4, indicating the calves had developed BRD during the marketing process before arrival.

*Block 3.* In the spring (Block 3; Table 2), there were no differences ( $P \geq 0.18$ ) in body weight or ADG during the receiving period, which is similar to our observations for fall and winter groups. Although, weight of the steers at the onset of grazing did not differ ( $P = 0.62$ ), steer weight was greater ( $P \leq 0.05$ ) for implanted steers on d 76, and during the entire grazing period (d 42 to 125;  $P = 0.02$ ). Likewise, overall ADG from d 0 to 125 was greater ( $P \leq 0.01$ ) for implanted steers compared to negative control. Similar to Blocks 1 and 2, ADG was lower than what may be expected during the early portion of the grazing period (d 42 to 76), averaging 1.7 lb/d across all treatments. In this first grazing period of the spring, steers implanted on d 0 and d 28 tended ( $P \leq 0.08$ ) to gain weight 0.37 lb/d faster than control, while ADG of steers implanted on d 14 was 0.62 lb/d greater ( $P \leq 0.01$ ) than control. Average daily gain during the final grazing period did not differ ( $P = 0.88$ ), which may be a function of reduced forage quality commonly encountered during the latter stages of wheat graze out associated with the increase in forage maturity and fiber content, thereby reducing forage digestibility and dietary energy intake. Over the entire grazing period in the spring, implanted steers gained an average of 0.21 lb/d more ( $P = 0.02$ ) than non-implanted steers. Daily gain of implanted

steers was also 0.18 lb/d greater ( $P = 0.01$ ) than control when calculated across both receiving and grazing periods (d 0 to 125). In contrast to poor overall health observed for Blocks 1 and 2, no calves in Block 3 were identified as having signs indicative of BRD. Therefore, statistical analysis of morbidity for the spring calves was not performed.

*Serology and Hematology.* Response to modified-live virus vaccination, as indicated by BVDV antibody titer concentration, was affected as expected by day ( $P < 0.001$ ; Table 3), but was not impacted by treatment ( $P = 1.00$ ; Table 4) nor was there a treatment  $\times$  day interaction ( $P = 0.88$ ) observed. This would suggest that a growth implant administered concurrent with vaccination did not impact the efficacy of either procedure.

There were effects of day of sampling on blood measurements taken during the receiving period (Table 3). These have been observed previously in studies with high-risk cattle. There were no main effects of implantation timing on any of the blood constituents measured (Table 4). The only blood measurement for which there was a treatment  $\times$  day interaction was the number of monocytes ( $P = 0.07$ ; Fig. 1).

## Implications

Although weight gains were not different during the receiving period, overall performance during the entire 120-day ownership period was increased similarly for implanted calves regardless of implant timing. Delaying implantation until later in the receiving period may result in improved performance during the later stages of an ownership period consisting of approximately 120 days. Health and vaccine response were not impacted by administration of a hormonal growth implant during initial processing or when the procedure was delayed until day 14 or 28. Under the conditions of the present study, there was no benefit to delaying administration of a growth implant.

**Table 1. Effect of growth implant and implant timing in stocker calves during the fall and winter (Block 1 and 2) on health and performance.**

Item	Treatment <sup>†</sup>				SE	P-value
	Control	Implant 0	Implant 14	Implant 28		
Bodyweight, lb						
Initial <sup>‡</sup>	442	448	447	450	5.9	0.79
Day 14	480	492	480	489	6.8	0.52
Day 28	511	526	508	520	7.1	0.25
Day 42 <sup>§</sup>	542	557	547	549	10.8	0.61
Day 63 <sup>¶</sup>	562	580	575	580	8.5	0.36
Day 91 <sup>#</sup>	629 <sup>a</sup>	670 <sup>b</sup>	660 <sup>b</sup>	664 <sup>b</sup>	9.1	0.10
Final BW <sup>††</sup>	689 <sup>a</sup>	733 <sup>b</sup>	725 <sup>b</sup>	737 <sup>b</sup>	9.2	<0.01
Average daily gain, lb/day						
D 0 to 14	2.72	3.10	2.39	2.72	0.26	0.16
D 14 to 28	2.19	2.47	2.03	2.29	0.26	0.27
D 28 to 42	2.23	2.18	2.78	2.04	0.67	0.34
Receiving average daily gain <sup>‡‡</sup>	2.38	2.58	2.40	2.35	0.22	0.28
D 42 to 63	0.95 <sup>a</sup>	1.17 <sup>ab</sup>	1.33 <sup>b</sup>	1.41 <sup>b</sup>	0.40	0.01
D 63 to 91	2.39 <sup>a</sup>	3.15 <sup>b</sup>	3.04 <sup>b</sup>	3.07 <sup>b</sup>	0.73	< 0.01
D 91 to 120	2.11 <sup>a</sup>	2.23 <sup>a</sup>	2.32 <sup>a</sup>	2.60 <sup>b</sup>	0.20	< 0.01
Grazing average daily gain <sup>§§</sup>	1.87 <sup>a</sup>	2.25 <sup>b</sup>	2.28 <sup>bc</sup>	2.41 <sup>c</sup>	0.10	< 0.01
Overall average daily gain <sup>¶¶</sup>	2.05 <sup>a</sup>	2.37 <sup>b</sup>	2.32 <sup>b</sup>	2.39 <sup>b</sup>	0.05	<0.01
Bovine respiratory disease treatment, %						
Treated once	89.7	96.0	93.8	98.4	8.7	0.52
Treated twice	24.1	12.4	25.8	27.8	12.9	0.30
Treated thrice	1.1	0.5	1.6	2.3	2.8	0.69
Days to first treatment	1.2	1.2	1.6	1.3	0.23	0.66

<sup>†</sup> Control = no growth implant administered; Implant 0 = growth implant administered on d 0; Implant 14 = growth implant administered on d 14; Implant 28 = growth implant administered on d 28.

<sup>‡</sup> Average body weight of steers on day -1 and 0 of receiving period.

<sup>§</sup> Bodyweight of steers at the end of the receiving period.

<sup>¶</sup> Bodyweight of steers on d 21 post receiving.

<sup>#</sup> Bodyweight of steers on d 49 post receiving.

<sup>††</sup> Average body weight of steers on d 119 and 120.

<sup>‡‡</sup> Average daily gain of steers from d 0 to 42.

<sup>§§</sup> Average daily gain of steers from d 42 to 120.

<sup>¶¶</sup> Average daily gain of steers from d 0 to 120.

<sup>a-c</sup> Least-squares means within a row with differing superscripts differ ( $P < 0.05$ ).



**Table 2. Effect of growth implant and implant timing in stocker calves during the spring (Block 3) on health and performance.**

Item	Treatment <sup>†</sup>				SE	P-value
	Control	Implant 0	Implant 14	Implant 28		
Bodyweight, lb						
Initial <sup>‡</sup>	532	532	533	530	6.0	0.99
Day 14	587	591	583	585	6.6	0.87
Day 28	611	620	617	613	7.4	0.84
Day 42 <sup>§</sup>	631	641	629	639	7.3	0.62
Day 76 <sup>¶</sup>	676 <sup>a</sup>	698 <sup>b</sup>	696 <sup>b</sup>	697 <sup>b</sup>	7.2	0.10
Final body weight <sup>#</sup>	773	798	794	795	8.5	0.13
Average daily gain, lb/d						
Day 0 to 14	3.87	4.21	3.61	3.92	0.19	0.27
Day 14 to 28	1.77	2.08	2.39	1.96	0.23	0.33
Day 28 to 42	1.36	1.48	0.91	1.85	0.44	0.54
Receiving average daily gain <sup>**</sup>	2.33	2.59	2.30	2.58	0.11	0.19
D 42 to 76	1.34 <sup>a</sup>	1.69 <sup>b</sup>	1.97 <sup>b</sup>	1.72 <sup>b</sup>	0.12	<0.01
D 76 to 125	1.97	2.05	1.98	1.99	0.88	0.88
Grazing average daily gain <sup>##</sup>	1.71 <sup>a</sup>	1.90 <sup>b</sup>	1.98 <sup>b</sup>	1.88 <sup>b</sup>	0.06	0.02
Overall average daily gain <sup>§§</sup>	1.93 <sup>a</sup>	2.13 <sup>b</sup>	2.09 <sup>b</sup>	2.12 <sup>b</sup>	0.05	0.01

<sup>†</sup> Control = no growth implant administered; Implant 0 = growth implant administered on d 0; Implant 14 = growth implant administered on d 14; Implant 28 = growth implant administered on d 28.

<sup>‡</sup> Average body weight of steers on day -1 and 0 of receiving period.

<sup>§</sup> Bodyweight of steers at the end of the receiving period.

<sup>¶</sup> Bodyweight of steers on day 34 post receiving.

<sup>#</sup> Average BW of steers on day 124 and 125.

<sup>\*\*</sup> Average daily gain of steers from day 0 to 42.

<sup>##</sup> Average daily gain of steers from day 42 to 125.

<sup>§§</sup> Average daily gain of steers from day 0 to 125.

<sup>a-b</sup> Least-squares means within a row with differing superscripts differ ( $P < 0.05$ ).

**Table 3. Effect of day of sampling of stocker calves on serology and hematology during the receiving period.**

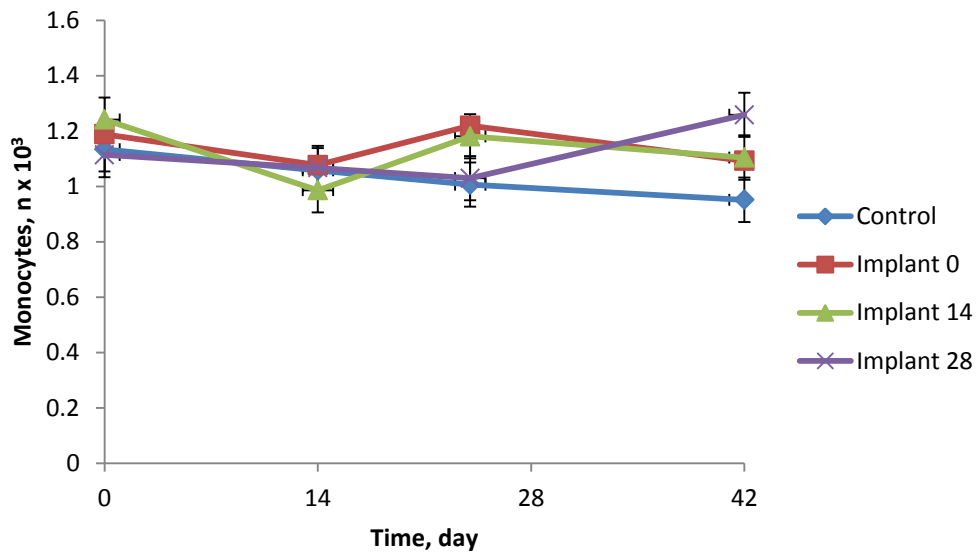
Item	Day				SE	P-value
	0	14	28	42		
BVDV antibody titer, log <sub>2</sub>	3.36 <sup>a</sup>	4.11 <sup>b</sup>	4.82 <sup>c</sup>	5.11 <sup>c</sup>	0.462	<0.001
White blood cells, n × 10 <sup>3</sup> /μL	9.4	9.5	9.6	10.2	0.40	0.26
Neutrophils, n × 10 <sup>3</sup> /μL	2.49	2.22	2.15	2.22	0.220	0.42
Lymphocytes, n × 10 <sup>3</sup> /μL	5.63	6.06	6.45	6.70	0.489	0.15
Monocytes, n × 10 <sup>3</sup> /μL	1.17 <sup>b</sup>	1.05 <sup>a</sup>	1.11 <sup>ab</sup>	1.10 <sup>ab</sup>	0.040	0.04
Eosinophils, n × 10 <sup>3</sup> /μL	0.05 <sup>a</sup>	0.12 <sup>b</sup>	0.12 <sup>b</sup>	0.13 <sup>b</sup>	0.018	<0.001
Basophils, n × 10 <sup>3</sup> /μL	0.09 <sup>ab</sup>	0.08 <sup>a</sup>	0.10 <sup>b</sup>	0.08 <sup>a</sup>	0.007	0.03
Neutrophil:Lymphocyte	0.72 <sup>b</sup>	0.65 <sup>ab</sup>	0.51 <sup>a</sup>	0.49 <sup>a</sup>	0.089	0.05
Red blood cells, n × 10 <sup>6</sup> /μL	10.41 <sup>b</sup>	10.06 <sup>a</sup>	10.12 <sup>a</sup>	10.10 <sup>a</sup>	0.114	<0.001
Hemoglobin, g/dL	12.7 <sup>c</sup>	12.3 <sup>a</sup>	12.5 <sup>b</sup>	12.6 <sup>bc</sup>	0.12	<0.001
Hematocrit, %	37.6 <sup>c</sup>	35.6 <sup>a</sup>	36.2 <sup>b</sup>	36.4 <sup>b</sup>	0.35	<0.001
Platelets, K/μL	589 <sup>a</sup>	809 <sup>b</sup>	604 <sup>a</sup>	565 <sup>a</sup>	43.7	<0.001

<sup>a-c</sup> Least-squares means within a row with differing superscripts differ ( $P \leq 0.05$ ).

**Table 4. Effect of implant and implant timing of stocker calves on serology and hematology during the receiving period.**

Item	Treatment <sup>†</sup>				SE	P-value
	Control	Implant 0	Implant 14	Implant 28		
BVDV antibody titer, log <sub>2</sub>	4.25	4.43	4.25	4.46	0.808	1.00
White blood cells, n × 10 <sup>3</sup> /μL	10.2	9.6	9.3	9.8	0.54	0.69
Neutrophils, n × 10 <sup>3</sup> /μL	2.23	2.37	2.20	2.29	0.256	0.97
Lymphocytes, n × 10 <sup>3</sup> /μL	6.74	5.97	5.81	6.32	0.533	0.62
Monocytes, n × 10 <sup>3</sup> /μL	1.04	1.14	1.13	1.12	0.044	0.32
Eosinophils, n × 10 <sup>3</sup> /μL	0.09	0.11	0.12	0.11	0.020	0.84
Basophils, n × 10 <sup>3</sup> /μL	0.08	0.09	0.09	0.08	0.008	0.82
Neutrophil:Lymphocyte	0.58	0.62	0.59	0.58	0.077	0.98
Red blood cells, n × 10 <sup>6</sup> /μL	10.29	10.16	10.28	9.96	0.196	0.62
Hemoglobin, g/dL	12.6	12.4	12.7	12.4	0.20	0.56
Hematocrit, %	36.8	36.2	36.9	36.1	0.54	0.62
Platelets, K/ μL	662	602	685	618	53.4	0.67

<sup>†</sup> Control = no growth implant administered; Implant 0 = growth implant administered on d 0; Implant 14 = growth implant administered on d 14; Implant 28 = growth implant administered on d 28.



**Fig. 1. Effect of growth implant and implant timing on number of monocytes (treatment × day, P = 0.07). Control = no growth implant administered; Implant 0 = growth implant administered on d 0; Implant 14 = growth implant administered on d 14; Implant 28 = growth implant administered on d 28.**

# Effect of timing of insemination with sorted semen on subsequent pregnancy rate in postpartum beef cows synchronized with a modified 14-day progesterone protocol

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## Story in Brief

This study investigated the effect of time of insemination on subsequent pregnancy rate when using X-chromosome sorted semen. Also evaluated was the effect of prostaglandin injection on d 7 of a modified 14-d progesterone protocol on estrous response. Over a two-year period, Angus-based primiparous (n = 74) and multiparous (n = 264) beef cows were assigned to treatment groups based on cyclicity, parity, weight, body condition and days postpartum. Treatment 1 (Control) cows received a controlled internal drug release (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<sub>2α</sub> (PGF) on d 23. Treatment 2 (D7PGF) cows received the same synchronization treatment, except an additional dose of prostaglandin was given on d 7 of CIDR treatment. All cows were observed for estrus over an 84-h period and inseminated with X-chromosome sorted semen between 9 and 24 h after detected estrus. Ten days later, all cows were exposed to fertile bulls for 45 days. Ultrasonography was used to determine pregnancy status of cows ~45 d after insemination and again 45 to 55 d after bull removal. Percentage of cows exhibiting estrus did not differ ( $P = 0.33$ ) at 76.5% and 71.2% for treatments 1 and 2, respectively. Pregnancy rates after artificial insemination (AI) with sorted semen were similar ( $P = 0.64$ ) at 63.3% and 66.7% for treatments 1 and 2, respectively. No differences ( $P = 0.98$ ) were detected in AI pregnancy rates when insemination occurred at intervals from 9 to 24 h after detected estrus. At the end of the breeding season, seasonal pregnancy rates were also similar ( $P = 0.74$ ) at 83.3% and 84.9% for cows in treatments 1 and 2, respectively. Results demonstrate the 14-d CIDR based protocol resulted in 70% or more of lactating beef cows expressing estrus within a 24 h period, and acceptable pregnancy rates can be achieved in lactating beef cows when using sorted semen over a range of insemination times.

## Introduction

Synchronization of cows with 14-d controlled internal drug release (CIDR) progesterone treatment, followed by gonadorelin (GnRH) on d 16 and prostaglandin F<sub>2α</sub> (PGF) on d 23 has resulted in good estrous response and artificial insemination (AI) pregnancy rates (Powell et al., 2011). Long-term progesterone treatment in cows that do not have a functional corpus luteum results in development of a persistent dominant follicle that should ovulate in response to GnRH on d 16, resulting in synchronized follicular growth and more consistent estrus after PGF is given on d 23. However, cyclic cows with a functional corpus luteum may not develop a persistent follicle due to higher progesterone concentrations. An objective of this study was to determine if the estrous synchronization protocol described above might be improved by the addition of PGF on d 7 of the CIDR treatment, to regress any corpus luteum present and insure a persistent follicle will develop that could respond to GnRH. Also, a trend for higher pregnancy rates has been noted when insemination with sorted semen is delayed until about 16 to 18 h after detected estrus (Rorie et al., 2012). Therefore, a second objective was to further evaluate the effect of time of insemination after onset of estrus on pregnancy rate when using sorted semen.

## Materials and Methods

During the fall breeding season of 2011 and 2012, Angus-cross, multiparous (n = 264) and primiparous (n = 74) lactating beef cows at the University of Arkansas beef research station at Savoy were randomly and equally distributed into two treatment groups based on cyclicity, weight, body condition and days postpartum (Table 1). Treatment 1 (Control) cows received a CIDR progesterone insert

(Eazi-Breed, Zoetis Inc., Florham Park, N.J.) 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 (D7PGF) 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 (Estroject, Rockway, Inc., Spring Valley, Wis.) was placed on all cows at the time of PGF on d 23. Cows were observed for estrus for 84 h after PGF, and those exhibiting estrus were inseminated with X-chromosome sorted semen at intervals from 9 to 24 h after onset of estrus. Ten days after the estrous detection period, all cows were exposed to fertile bulls for 45 days. Transrectal ultrasonography was used to determine AI pregnancy status of cows at ~45 d of gestation and again 45 to 55 days after bull removal of seasonal pregnancy rate. Differences in fetal crown-rump length were used to determine if pregnancies resulted from artificial insemination or subsequent matings. Percentage data for estrous response and pregnancy rate were evaluated using the Chi-Square analysis. All other data were evaluated by analysis of the variance. Data were initially analyzed for year, treatment and their interaction. No significant year or treatment × year interaction was detected ( $P \geq 0.35$ ), so data for both years were combined for analysis.

## Results and Discussion

The estrous synchronization protocol used in this study was based on the assumption that long-term CIDR treatment would result in development of a large persistent follicle capable of ovulating in response to GnRH given a couple of 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

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the GnRH treatment will be ineffective. In these cows, injection of PGF on d 7 of the CIDR treatment should regress the corpus luteum and insure that a persistent follicle develops that 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.

The percentage of cows exhibiting estrus did not differ ( $P = 0.33$ ) between treatments (76.5% and 71.2% for control and D7PGF treatments, respectively (Table 2). The estrous response was acceptable considering that at the start of estrous synchronization only about two-thirds of the cows were cyclic (Table 1). Cows that are not cyclic would not have had a functional corpus luteum and would not respond to PGF given on d 7 of CIDR treatment. This might explain why no treatment differences were detected in estrous response to synchronization. The mean interval from PGF treatment on d 23 to detected estrus was 3 h longer ( $P = 0.03$ ) for cows in the D7PGF treatment. Within 48 h of PGF treatment, 25% of the control cows were observed in estrus compared to 6% in the D7PGF treatment. During a 24 h period (48 h to 72 h after PGF) 89% of the cows in the PGF group expressed estrus compared with 69% of the cows in the control group. While the percentage of cows expressing estrus did not differ among treatments, the D7PGF treatment appeared to have resulted in better synchrony of estrus.

Pregnancy rates after AI with sorted semen were similar ( $P = 0.64$ ) at 63.3% and 66.7% for treatments 1 and 2, respectively. Pregnancy rates from sorted semen usually are somewhat lower than that achieved with unsorted semen, likely due to the reduced number of sperm per insemination dose and potential damage to sperm during the sorting process. However, these results demonstrate that a pregnancy rate in excess of 60% can be achieved with sorted semen. No differences ( $P = 0.98$ ) were detected in AI pregnancy rates when insemination occurred at intervals from 9 to 24 h after detected estrus (Fig. 1).

Previously, our preliminary data suggested pregnancy rates might be improved by delaying insemination a few hours later than the usual 12 h after onset of estrus, when using sorted semen. In dairy heifers, pregnancy rate is highest when insemination with sorted semen occurs from 16 to 24 h after onset of estrus (Filho

et al., 2010). In the current study, the majority of beef cows were inseminated with sorted semen from 15 to 21 h after detected estrus, resulting in a pregnancy rate of about 65%. Although this data does not show any effect of time of insemination on pregnancy rate, it is certainly acceptable to delay insemination until 15 to 20 h after onset of estrus. Semen quality is known to vary among bulls. Delaying time of insemination reduces the time sperm must survive the cows' reproductive tract before ovulation, and could improve pregnancy rates in cases where semen quality is less than optimal. Synchronization treatment had no effect ( $P = 0.74$ ) on overall pregnancy rate. At the end of the breeding season, seasonal pregnancy rates were 83.3% and 84.9% for cows in the control and D7PGF treatments, respectively.

## Implications

Results demonstrate that an estrous synchronization protocol consisting of CIDR for 14 d, GnRH on d 16 and PGF on d 23 was effective in synchronizing  $\geq 70\%$  of lactating beef cows within a 24 h period. Acceptable pregnancy rates can be achieved in lactating beef cows when using sorted semen over a range of insemination times.

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**Table 1. Distribution of beef cows across synchronization treatments.**

Parameter	Synchronization Treatment		P value
	Control	D7PGF	
Weight (lbs)	1160.1 ± 14.6	1153.3 ± 14.6	0.74
Body condition (BCS)	5.23 ± 0.1	5.22 ± 0.1	0.97
Post partum interval (d)	57.6 ± 1.4	58.8 ± 1.4	0.53
Cows cyclic at synchronization	89/132 (67.4%)	86/132 (65.2%)	0.88

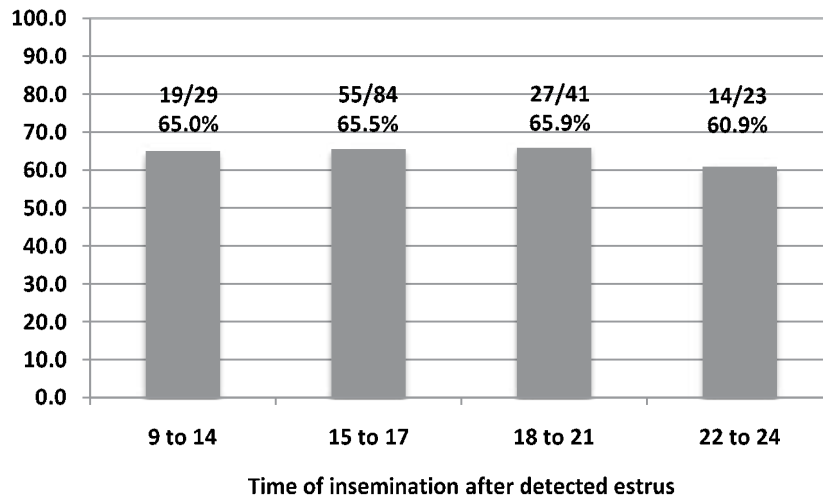
**Table 2. Effect of synchronization treatment on estrous response and pregnancy rates.**

Parameter	Control	D7PGF	P value
Estrous response	101/132 (76.5%)	94/132 (71.2%)	0.33
Interval, PGF <sup>†</sup> to estrus (h)	54.3 ± 1.0 <sup>a</sup>	57.4 ± 1.0 <sup>b</sup>	0.03
AI preg. rate, sorted Semen <sup>‡</sup>	57/90 (63.3%)	58/87 (66.7%)	0.64
Season preg. rate	110/132 (83.3%)	112/132 (84.9%)	0.74

<sup>†</sup> PGF = prostaglandin F<sub>2α</sub>.

<sup>‡</sup> Excludes 7 cows in the control group and 3 cows in the D7PGF group that were inseminated with unsorted semen.

<sup>a,b</sup> Means within rows with different superscripts differ ( $P = 0.03$ ).



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

# Intake, digestibility and ruminal fermentation characteristics of cows limit-fed co-product commodity feeds

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## Story in Brief

In years of adverse conditions, especially drought years, the economics of buying and shipping hay often does not justify its nutritional content. Also, during periods of limited nutrient availability, the cost of buying and shipping hay often is not feasible for many small-scale family farm operations. It is possible that co-product commodities are the most economical way to maintain cows in this situation. Therefore, the objective was to determine the effects of limit-feeding co-product commodity feeds on ruminal fermentation and overall digestibility. Eight ruminally cannulated cows (1480 ± 70.5 lb initial body weight) were allocated randomly to one of four treatments: limit-fed soybean hulls (LSH), limit-fed distillers' dried grains with solubles (LDG), limit-fed a mixture of soybean hulls and distillers' dried grains with solubles (MIX), or provided ad libitum access to hay (HAY). Limit-fed diets were formulated to meet the metabolizable energy requirements of an 11-month post-partum mature beef cow. Dietary co-product amounts were increased over a 14-day period. This was followed by a 14-day adaptation to diet and facilities and 5 days of total fecal collections. On the final day of fecal collections, rumen fluid was sampled immediately prior to feeding and 2, 4, 6, 8, 10 and 12 hours post-feeding for measurement of rumen volatile fatty acid and ammonia concentrations. Although dry matter intake was not different ( $P > 0.10$ ) among treatments, dry matter digestibility was greatest ( $P < 0.05$ ) by cows offered limit-fed soybean hulls and a limit-fed mixture of soybean hulls with distillers' dried grains with solubles, intermediate ( $P < 0.05$ ) by cows offered limit-fed distillers' dried grains with solubles, and lowest ( $P < 0.05$ ) for those offered hay. An interaction of treatment and time ( $P < 0.05$ ) was observed for rumen pH, acetate, propionate and butyrate concentrations, acetate to propionate ratio and ammonia-N concentrations. Based on these data, co-product commodities may be used for maintenance of cows without adverse effects on digestive and fermentative performance.

## Introduction

Limit feeding is a nutritional strategy that is often employed in feedlot diets as "restricted feeding" and in grower programs as "programmed feeding" (Galyean, 1999). Loerch (1996) employed a limit-feeding strategy for gestating beef cows, replacing hay in the diet with corn as an economically sound alternative in years of decreased hay production. In a similar study, Dreidger and Loerch (1999) observed that limit-fed corn tended to increase dry matter digestibility of the diet. However, little information is available as to the effect of limit or programmed feeding on rumen fermentation characteristics. Therefore, the objective in this study was to determine the effects of limit feeding soybean hulls, distillers' dried grains with solubles, or a combination of the two on ruminal fermentation and overall digestibility.

## Materials and Methods

Eight ruminally cannulated cows (1480.3 ± 370.08 lb initial body weight; 9 years of age) were used in a 2-period study to evaluate 4 different diets. In each period, cows were stratified by body weight and allocated randomly to one of four treatments (2 cows/diet): limit-fed soybean hulls (LSH), limit-fed distiller's dried grains with solubles (LDG), limit-fed an isoenergetic mixture of soybean hulls and distiller's dried grains with solubles (MIX), or provided ad libitum access to hay (HAY). Diets were formulated to meet the metabolizable energy requirements of a pregnant 11-month post-partum mature beef cow based on the published nutritional composition of each feedstuff. Ground limestone was added to the LDG and MIX diets to equalize diet Ca concentrations (NRC,

2000). Cows receiving limit-fed diets were offered 2 lb hay daily for roughage consumption. Cows on the HAY treatment were offered 2 lb of the MIX diet to ensure adequate protein intake.

In order to allow cows to adapt to their respective diets, they were offered ad libitum access to hay from large round bales for the first 7 days as a group, separated each morning at approximately 8:00 AM, and offered increasing levels of their respective supplements. Once their daily supplement amount was reached, the amount of time cows had access to hay was reduced over the following 7 days. Following the initial adjustment period, cows were moved to an enclosed barn with 10 × 14-ft stalls fitted with smooth rubber flooring. Diets were offered at 8:00 AM daily for a 14-day adaptation period. Cows were allowed a two-hour period to consume concentrates followed by offering of hay as determined by dietary treatment. Rejected feed was collected from feed bunks prior to the 8:00 AM feeding. Animals had ad libitum access to water throughout the trial, and a commercial trace mineral supplement (0.10 lb) was mixed with the concentrate diet daily.

Following the adaptation period, total fecal collections occurred for a 5-day period. Feces were collected throughout the day and placed in trash cans lined with plastic liners. Feces were weighed at 8:00 AM daily, mixed in a mobile concrete mixer, and a subsample taken for chemical analysis. Hay, reject feed, and fecal samples were dried to a constant weight at 122 °F for dry matter (DM) determination. Representative samples were composited and ground to 0.04 inches using a Wiley mill (Arthur H. Thomas, Philadelphia, Pa.).

On the final day of fecal collections, rumen fluid was sampled immediately prior to feeding and 2, 4, 6, 8, 10 and 12 hours post feeding. Rumen contents were taken from four different regions of the rumen and fluid was strained through eight layers of cheesecloth.

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Rumen fluid pH was measured immediately, and samples of rumen fluid were preserved for later analysis of volatile fatty acids (VFA) and ammonia.

After the first treatment period, cows were co-mingled in a drylot and offered ad libitum access to hay from large round bales along with 2 lb of the MIX supplement for 4 weeks. After this period, cows were re-allocated randomly to one of the four diets with the stipulation that they did not receive the same diet as they were offered in the first period. The adaptation and collection periods occurred as previously described. Data were analyzed using the mixed procedure of SAS (SAS Institute, Inc., Cary, N.C.) where animal served as the experimental unit and time was used as a repeated measure for pH, VFA and ammonia-nitrogen (-N). For analysis of dry matter intake and digestibility, the model included treatment and the random variables included cow, period and cow within treatment by period.

## Results and Discussion

Dry matter intake (DMI) was not different among treatments ( $P = 0.33$ ; Table 1). Dry matter digestibility was greatest for MIX and LSH followed by LDG with the lowest DM digestibility occurring for HAY ( $P < 0.05$ ). Digestible DMI tended ( $P < 0.10$ ) to be greater from LSH and MIX compared with LDG and HAY.

Effects of treatment, time and their interaction were detected ( $P < 0.05$ ) for ruminal pH. Immediately prior to feeding, ruminal pH from cows offered LDG was greater ( $P < 0.05$ ) compared to cows offered HAY and LSH but not different from cows offered MIX (Table 2). At 2 hours post feeding, and continuing through 8 hours post feeding, ruminal pH was generally greater from cows offered HAY compared with those offered the limit-fed concentrate diets. The exceptions were that ruminal pH from cows offered HAY were not different ( $P > 0.10$ ) from those offered LSH at 2 hours post feeding, and those offered LDG at 6 and 8 hours post feeding. Ruminal pH did not differ ( $P < 0.10$ ) among treatments at 10 and 12 hours post feeding. Although the use of commodity feeds appeared to result in a decrease in rumen pH, rumen digestive function did not appear to be affected. Total VFA concentrations were different among treatments and across sampling times ( $P < 0.05$ ), but the treatment by time interaction was not significant. Total concentrations of VFA for LSH were greater ( $P < 0.05$ ) than those from MIX, HAY and LDG (Table 3).

Effects of treatment, time and their interaction were detected ( $P < 0.05$ ) for acetate, propionate, and butyrate concentrations and the acetate to propionate ratio. Acetate concentrations were greater ( $P < 0.05$ ) from HAY and LSH compared to LDG at each sampling time. Acetate concentrations from cows offered MIX were not different ( $P > 0.10$ ) from those by cows offered HAY and LSH immediately prior to feeding, and at 8, 10, and 12 hours post feeding, but were different ( $P < 0.05$ ) from that of cows offered HAY and LSH at 2, 4, and 6 hours post feeding.

Greater propionate concentrations were observed from cows offered LDG ( $P < 0.05$ ) than those by cows offered the other diets at every sampling time. Propionate concentrations from cows offered MIX were greater ( $P < 0.05$ ) than those by cows offered HAY and LSH at 4 hours post feeding, but not at the remaining sampling

times. Butyrate concentrations did not differ ( $P > 0.10$ ) among treatments immediately prior to feeding, but were greater ( $P < 0.05$ ) from cows offered LDG and MIX at 2, 4, and 6 hours compared with cows offered HAY and LSH. Butyrate concentrations at 2, 4, 6, 8, and 10 hours after feeding were greater for cows offered LDG ( $P < 0.05$ ) than for cows offered LSH and HAY. At 12 hours post feeding, concentrations of butyrate were greater for cows offered LDG and MIX ( $P < 0.05$ ) than those by cows offered HAY.

Trends observed for the acetate:propionate ratios were similar to those observed with acetate. Cows offered HAY and LSH had the greatest ( $P < 0.05$ ) acetate:propionate ratios throughout the first 8 hours following feeding, and cows offered LDG had the lowest ( $P < 0.05$ ) acetate:propionate ratios at every sampling time.

In addition to the difference in total concentration of VFA, the differences observed for each of the VFA and the acetate to propionate ratios add to available knowledge on energy status of animals under a limit-feeding system. Little information is available as to the effects of limit-fed diets on fermentation characteristics by ruminant animals.

Effects of treatment, time and their interaction were significant ( $P < 0.05$ ) for the concentrations of ruminal ammonia-N (Table 4). Immediately prior to feeding, ammonia-N concentrations were greater ( $P < 0.05$ ) from cows offered LDG followed by those offered MIX (Table 5). Rumen ammonia-N concentrations from cows offered LDG and MIX were greater ( $P < 0.05$ ) compared to those offered HAY and MIX at 2 hour post feeding, as well as from cows offered LSH at 4 and 6 hours post feeding. Ammonia-N concentrations were also greater for those cows offered LDG ( $P < 0.05$ ) compared to cows offered the other diets at 8 and 10 hours after feeding.

## Implications

No differences were observed for dry matter intake, but dry matter digestibility and digestible dry matter intake were greater for limit-fed diets as opposed to hay. Limit-feeding commodity feeds did lower rumen pH but digestive function did not seem to be compromised. The use of limit-fed soybean hulls greatly increased total volatile fatty acids, and all commodity feeds increased propionate and butyrate concentrations. Limit-fed commodities, especially distiller's dried grains with solubles, were observed to increase rumen concentrations of ammonia-N and propionate. Based on these data, commodity feeds can be limit fed to cows without adverse effects on digestive or fermentative function of the rumen.

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**Table 1. Intake and digestibility by cows limit-fed co-product commodity feeds.**

Item <sup>†</sup>	HAY <sup>‡</sup>	LSH	LDG	MIX	SEM <sup>§</sup>
DMI, % BW	1.3	1.3	1.1	1.2	0.12
DMD, %	55.2 <sup>c</sup>	71.8 <sup>a</sup>	69.6 <sup>b</sup>	77.0 <sup>a</sup>	1.52
Digestible DMI, % BW	0.70 <sup>e</sup>	0.95 <sup>d</sup>	0.75 <sup>e</sup>	0.90 <sup>d</sup>	0.071

<sup>a,b,c</sup> Means within a row differ ( $P < 0.05$ ).

<sup>d,e</sup> Means within a row differ ( $P < 0.10$ ).

<sup>†</sup> DMI = dry matter intake; DMD = dry matter digestibility.

<sup>‡</sup> HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distiller's dried grains with solubles; MIX = limit-fed an isoenergetic mix of soybean hulls and distiller's dried grains with solubles.

<sup>§</sup> Pooled standard error of the mean.

**Table 2. Rumen pH by cows limit-fed co-product commodity feeds.**

Time, h	HAY <sup>†</sup>	LSH	LDG	MIX	SEM <sup>‡</sup>
0	6.66 <sup>b</sup>	6.48 <sup>b</sup>	7.26 <sup>a</sup>	6.87 <sup>ab</sup>	0.200
2	6.77 <sup>a</sup>	6.36 <sup>ab</sup>	6.29 <sup>b</sup>	6.23 <sup>b</sup>	0.200
4	6.83 <sup>a</sup>	5.84 <sup>b</sup>	5.95 <sup>b</sup>	6.17 <sup>b</sup>	0.200
6	6.35 <sup>a</sup>	5.49 <sup>c</sup>	6.04 <sup>ab</sup>	5.86 <sup>bc</sup>	0.200
8	6.60 <sup>a</sup>	5.79 <sup>c</sup>	6.22 <sup>ab</sup>	6.04 <sup>bc</sup>	0.200
10	6.23	5.81	6.13	6.02	0.200
12	6.06	5.96	6.18	6.00	0.200

<sup>a,b,c</sup> Means within a row differ ( $P < 0.05$ ).

<sup>†</sup> HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distiller's dried grains with solubles; MIX = limit-fed an isoenergetic mix of soybean hulls and distiller's dried grains with solubles.

<sup>‡</sup> Pooled standard error of the mean.



**Table 3. Rumen volatile fatty acids by cows limit fed co-product commodity feeds.**

	Time, h	HAY <sup>†</sup>	LSH	LDG	MIX	SEM <sup>‡</sup>
Total VFA <sup>§</sup> , mM		83.6 <sup>b</sup>	107.8 <sup>a</sup>	66.4 <sup>b</sup>	85.8 <sup>b</sup>	6.16
Acetate, % of Total VFA	0	70.1 <sup>a</sup>	68.5 <sup>a</sup>	56.5 <sup>b</sup>	68.6 <sup>a</sup>	2.07
	2	67.8 <sup>a</sup>	69.5 <sup>a</sup>	47.3 <sup>c</sup>	60.6 <sup>b</sup>	2.07
	4	69.7 <sup>a</sup>	69.2 <sup>a</sup>	46.3 <sup>c</sup>	61.7 <sup>b</sup>	2.07
	6	68.8 <sup>a</sup>	68.3 <sup>a</sup>	48.0 <sup>c</sup>	63.8 <sup>b</sup>	2.07
	8	68.3 <sup>a</sup>	67.6 <sup>a</sup>	49.6 <sup>b</sup>	65.2 <sup>a</sup>	2.07
	10	67.9 <sup>a</sup>	67.8 <sup>a</sup>	52.7 <sup>b</sup>	65.3 <sup>a</sup>	2.07
	12	68.2 <sup>a</sup>	67.7 <sup>a</sup>	54.7 <sup>b</sup>	66.9 <sup>a</sup>	2.07
Propionate, % of Total VFA	0	18.1 <sup>b</sup>	17.9 <sup>b</sup>	26.4 <sup>a</sup>	17.4 <sup>b</sup>	1.57
	2	19.6 <sup>b</sup>	19.0 <sup>b</sup>	32.4 <sup>a</sup>	22.5 <sup>b</sup>	1.57
	4	18.4 <sup>c</sup>	19.1 <sup>c</sup>	35.9 <sup>a</sup>	22.8 <sup>b</sup>	1.57
	6	18.6 <sup>b</sup>	19.6 <sup>b</sup>	35.2 <sup>a</sup>	22.4 <sup>b</sup>	1.57
	8	19.3 <sup>b</sup>	19.5 <sup>b</sup>	33.7 <sup>a</sup>	21.8 <sup>b</sup>	1.57
	10	19.9 <sup>b</sup>	19.4 <sup>b</sup>	31.2 <sup>a</sup>	21.8 <sup>b</sup>	1.57
	12	19.4 <sup>b</sup>	18.9 <sup>b</sup>	30.1 <sup>a</sup>	21.0 <sup>b</sup>	1.57
Butyrate, % of Total VFA	0	7.5	8.6	8.4	8.6	0.97
	2	7.9 <sup>b</sup>	7.3 <sup>b</sup>	14.6 <sup>a</sup>	13.1 <sup>a</sup>	0.97
	4	7.7 <sup>b</sup>	7.6 <sup>b</sup>	13.3 <sup>a</sup>	12.1 <sup>a</sup>	0.97
	6	8.0 <sup>b</sup>	8.0 <sup>b</sup>	12.5 <sup>a</sup>	11.1 <sup>a</sup>	0.97
	8	8.3 <sup>b</sup>	8.4 <sup>b</sup>	12.3 <sup>a</sup>	10.0 <sup>ab</sup>	0.97
	10	8.2 <sup>b</sup>	8.3 <sup>b</sup>	11.9 <sup>a</sup>	10.0 <sup>ab</sup>	0.97
	12	7.8 <sup>b</sup>	8.5 <sup>ab</sup>	11.1 <sup>a</sup>	9.2 <sup>a</sup>	0.97
Acetate:propionate	0	4.0 <sup>a</sup>	4.0 <sup>a</sup>	2.1 <sup>b</sup>	3.9 <sup>a</sup>	0.26
	2	3.6 <sup>a</sup>	3.7 <sup>a</sup>	1.5 <sup>c</sup>	2.7 <sup>b</sup>	0.26
	4	3.9 <sup>a</sup>	3.7 <sup>a</sup>	1.4 <sup>c</sup>	2.7 <sup>b</sup>	0.26
	6	3.8 <sup>a</sup>	3.6 <sup>a</sup>	1.4 <sup>c</sup>	2.8 <sup>b</sup>	0.26
	8	3.6 <sup>a</sup>	3.6 <sup>a</sup>	1.5 <sup>c</sup>	2.9 <sup>b</sup>	0.26
	10	3.5 <sup>ab</sup>	3.6 <sup>a</sup>	1.7 <sup>c</sup>	3.0 <sup>b</sup>	0.26
	12	3.6 <sup>a</sup>	3.7 <sup>a</sup>	1.8 <sup>b</sup>	3.1 <sup>a</sup>	0.26

<sup>a,b,c</sup> Means within a row differ ( $P < 0.05$ ).

<sup>†</sup> HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distiller's dried grains with solubles; MIX = limit-fed an isoenergetic mix of soybean hulls and distiller's dried grains with solubles.

<sup>‡</sup> Pooled standard error of the mean.

<sup>§</sup> VFA = volatile fatty acids.

**Table 4. Rumen ammonia-N, ng/dL by cows limit fed co-product commodity feeds.**

Time, h	HAY <sup>†</sup>	LSH	LDG	MIX	SEM <sup>‡</sup>
0	7.77 <sup>bc</sup>	6.07 <sup>c</sup>	18.92 <sup>a</sup>	12.31 <sup>b</sup>	1.806
2	8.43 <sup>b</sup>	7.43 <sup>b</sup>	14.68 <sup>a</sup>	16.72 <sup>a</sup>	1.806
4	7.77 <sup>ab</sup>	3.23 <sup>b</sup>	11.59 <sup>a</sup>	11.78 <sup>a</sup>	1.806
6	5.38 <sup>ab</sup>	2.53 <sup>b</sup>	9.72 <sup>a</sup>	7.49 <sup>a</sup>	1.806
8	3.13 <sup>b</sup>	3.05 <sup>b</sup>	10.79 <sup>a</sup>	4.02 <sup>b</sup>	1.806
10	2.07 <sup>b</sup>	3.52 <sup>b</sup>	10.09 <sup>a</sup>	4.85 <sup>b</sup>	1.806
12	2.44 <sup>b</sup>	3.15 <sup>b</sup>	9.46 <sup>a</sup>	5.30 <sup>ab</sup>	1.806

<sup>a,b,c</sup> Means within a row differ ( $P < 0.05$ ).

<sup>†</sup> HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distiller's dried grains with solubles; MIX = limit-fed an isoenergetic mix of soybean hulls and distiller's dried grains with solubles.

<sup>‡</sup> Pooled standard error of the mean.

# Production characteristics and blood metabolites of gestating cows limit-fed soybean hulls

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## Story in Brief

In droughty years, forage for grazing and haying can become limited. The economics of buying and shipping hay often does not justify its nutrient content. Co-product commodities may be a more economical way to maintain a cowherd through this situation. Our objective was to determine if soybean hulls could be used to meet the majority of the energy demands for cows in late pregnancy along with limited hay consumption. Eighty-six gestating cows ( $1162 \pm 1.8$  lb initial body weight;  $4.3 \pm 0.03$  years of age) were allocated to 1 of 6 groups on December 6, 2012. Three of the groups were offered medium-quality bermudagrass hay, free choice. The three remaining groups were offered 14 lb soybean hulls daily and allowed access for 1 hour daily to a very poor-quality, mixed-grass hay harvested from Conservation Reserve Program land. Each group was housed in separate 5-acre dormant bermudagrass pastures. Cows remained on these diets for 68 days (until February 12, 2013). Upon calving, birth weight and dystocia scores were recorded. Representative bales were weighed to determine total hay offered. Differences in weight and body condition score, and changes in these measurements during the study did not differ between treatments ( $P \geq 0.31$ ). Calf birth weights, but not dystocia scores, were different between treatments (86 lb for ad libitum hay, 95 lb for limit-fed soybean hulls;  $P = 0.05$  and  $0.23$ , respectively). Based on this information, it appears that soybean hulls can be limit fed to cows to meet their energy requirements during late pregnancy without adverse effects on the cows or subsequent calves.

## Introduction

Limit feeding is a nutritional strategy that is often employed in feedlot diets as “restricted feeding” and in grower programs as “programmed feeding,” but not as commonly in cow-calf operations (Galyean, 1999). Loerch (1996) employed a limit-fed corn system for gestating beef cows, replacing most of the hay in the diet. At that time, a corn-based, limit-fed diet was more cost effective than feeding hay. Additionally, limit-feeding of gestating cows has been shown to decrease manure and nutrient output, offering an environmental incentive to the practice (Dreidger and Loerch, 1999). As corn prices have risen with the federal mandate for ethanolic fuels, program feeding corn may be more costly than other options. Co-product commodity feeds can also provide an economic alternative to hay in years of adverse climatic conditions. Our objective was to determine if soybean hulls could be used to meet the majority of the energy demands for cows in later pregnancy with access to hay limited to 1 hour daily.

## Materials and Methods

Eighty-six gestating Gelbvieh  $\times$  Angus cows ( $1162 \pm 16.5$  lb initial body weight;  $4.3 \pm 0.27$  years of age) were weighed and body condition score (BCS) assessed on November 28 and December 6, 2012, and the average of these measurements were used as the starting values. Cows were allocated randomly to 1 of 6 groups and groups were assigned randomly to 1 of 2 treatments. Each group was housed in a separate 5-acre, dormant bermudagrass [*Cynodon dactylon* (L.) Pers.] pasture with negligible available forage to graze. Three groups were offered medium-quality bermudagrass hay for ad libitum consumption throughout the study (HAY). The

remaining three groups were offered 14 lb/cow, daily, of pelleted soybean hulls (LSH). This level was calculated to meet the mean metabolizable energy requirement, assuming a minimum of 5 lb of hay consumption daily per cow. Cows in groups assigned to the LSH treatment were also allowed 1 hour access each morning to very poor quality mixed-grass hay harvested from Conservation Reserve Program (CRP) ground at the Pine Tree Research Station near Pine Tree, Ark. Cows remained on their respective diets for 68 days. Weights were taken and BCS assessed on days 39 and 68 to assess weight and BCS change. At calving, birth weight and dystocia scores were recorded.

Representative bales of each treatment were selected at random in the first feeding period and weighed to determine hay intake. Residual hay and hay waste were estimated visually at the end of the study. This amount was negligible because cows were forced to clean up old hay during the final days of the study. Representative hay samples were gathered at the time bales were placed in the respective pastures and were dried to a constant weight at 122 °F for dry matter (DM) determination. Samples were subsequently analyzed for percentages of neutral-detergent fiber (NDF; Table 1).

On days 1, 39 and 68, blood was collected via jugular venipuncture from each cow and analyzed for non-esterified fatty acid (NEFA) concentrations. Serum NEFA is a reliable indicator of body condition changes within an animal. An increase in serum NEFA concentration indicates a mobilization of body fat stores from a deficit in energy balance.

Data were analyzed using the mixed procedure of SAS (SAS Institute, Inc., Cary, N.C.) where pasture served as the experimental unit and time was a repeated measure for NEFA comparisons. Effects of treatment as well as cow age were tested in the model.

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## Results and Discussion

Initial body weight, interim and final body weights, as well as BCS at each of these dates, did not differ between treatments ( $P \geq 0.47$ ; Table 2). Body weight at all 3 time intervals did differ by age. When grouped by age (2-yr olds, 3-yr olds, > 3-yr olds), body weight increased with increasing age ( $P < 0.05$ ), and BCS was greater ( $P < 0.05$ ) for cows greater than 3 years of age than for 2- and 3-year old cows for day 0 and 68. Subsequent BCS at the time of calving did not differ among treatments ( $P = 0.34$ ) or age groups ( $P = 0.12$ ).

Changes in body weight across days 0, 39 and 68 did not differ between treatments ( $P \geq 0.31$ ) or age groups ( $P \geq 0.29$ ). Change in BCS across days 0, 39 and 68 did not differ between treatments ( $P \geq 0.27$ ). Change in BCS was different between day 0 and 39 ( $P < 0.05$ ) and tended to differ between day 0 and 68 ( $P < 0.10$ ).

Non-esterified fatty acids did not differ among treatments or age groups ( $P \geq 0.38$ ), and no interaction existed between treatment and sampling date ( $P = 0.47$ ). Serum NEFA concentrations differed among sampling dates ( $P < 0.05$ ) with the final measurement having greater ( $P < 0.05$ ) NEFA concentrations than either the initial or interim measurements.

Hay intake from LSH cows was not substantially decreased by 1 hour restricted access. Intake exceeded the estimation (5 lb/hd/day) used for diet formulations. Crude protein intake was greater than published requirements for both LSH and HAY. Intake of total digestible nutrients was greater than published requirements for LSH, but not for HAY. However, body weight and BCS changes did not differ between treatments, thus nullifying this effect.

Daily feed cost, expressed as dollars per cow, was \$ 0.61/day greater for HAY compared with LSH. Over the course of the study,

this would compute to a total saving per cow of \$42 (Table 3).

Calves born from LSH cows tended ( $P = 0.05$ ) to be 8.7 lb heavier at birth than those born to HAY cows. Dystocia scores for calving did not differ ( $P \geq 0.23$ ) among treatments or age groups.

## Implications

Performance of cow's limit-fed soybean hulls was similar to cows allowed ad libitum access to bermudagrass hay. Body weight and body condition score increased for both treatments and serum non-esterified fatty acid concentrations did not indicate an adverse effect of the limit-feeding strategy when compared to ad libitum hay. Additionally, limit-fed soybean hulls represented a \$42 saving per cow over the course of this study. Based on the data, it appears that soybean hulls can be limit-fed to cows in mid- to late gestation without adverse effects on cow or calf performance.

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**Table 1. Neutral-detergent fiber concentration of soybean hulls and hays offered to gestating cows.**

Item	Neutral Detergent Fiber (% DM)
Period 1	
Soybean hulls	63.16
Hay (Mississippi origin)	70.16
Hay (Pine tree station)	79.30
Period 2	
Soybean hulls	63.01
Hay (Mississippi origin)	73.45
Hay (Pine tree station)	83.69

**Table 2. Performance and serum non-esterified fatty acids by gestating cows limit-fed soybean hulls.**

Item		HAY <sup>†</sup>	LSH	SEM <sup>‡</sup>
Body weight, lb	0	1138	1139	19.1
	39	1230	1223	20.7
	68	1209	1218	21.0
	Change (0 to 68)	72	80	15.0
Body condition score	0	6.5	6.4	0.08
	39	6.6	6.7	0.09
	68	6.8	6.9	0.06
	Change (0 to 68)	0.4	0.5	0.08
NEFA <sup>§</sup> , µeq/dL	0	339.0	351.2	47.00
	39	308.2	398.7	47.00
	68	632.9	692.7	47.00
Calf birth weight		86 <sup>a</sup>	95 <sup>b</sup>	2.3
Dystocia score		0.0	0.2	0.13

<sup>a,b</sup> Means within a row differ ( $P < 0.10$ ).

<sup>†</sup> HAY = ad libitum hay; LSH = limit-fed soybean hulls.

<sup>‡</sup> Pooled standard error of the mean.

<sup>§</sup> NEFA = non-esterified fatty acids.

**Table 3. Intake and cost of hay vs. limit-fed soybean hulls for gestating cows.**

Item <sup>†</sup>	HAY <sup>‡</sup>	LSH
Hay intake, lb/day	31.8	14.6
Hay intake, % BW	2.7	1.3
Soybean hull intake, lb/day	0.0	14.0
Estimated CP intake <sup>§</sup> , lb/day	2.5	3.1
Estimated TDN intake <sup>§</sup> , lb/day	15.9	18.4
Daily feed cost, \$/cow	\$2.86	\$2.25
Total feed cost, \$/cow	\$195.00	\$153.00

<sup>†</sup> BW = body weight; CP = crude protein; TDN = total digestible nutrients.

<sup>‡</sup> HAY = ad libitum hay; LSH = limit-fed soybean hulls.

<sup>§</sup> Estimated according to published values (NRC, 2000).

# Balking behavior in cattle breed-type prevalence based on coat color and potential carcass implications

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## Story in Brief

Balking behavior in the cattle processing line can pose welfare issues as electric prod use to coerce forward movement is implemented. Temperament differences have been shown among breed-type categories, within breed-type categories, among crossbreds, and between genders. Objectives in this initial study were to determine if breed-type predominance, based on coat color or gender, has an effect on balking behavior, and if this behavior affects carcass economics. A total of 2,375 balking observations, on a scale of 1-5 by a single observer, were recorded at the entrance to the restrainer in a high-capacity processing plant. Balking score, coat color, and characteristic markings were correlated with mean pen weights and dressing percentages. Descending order of balking mean by breed-types during two consecutive day observations is Holsteins, red-mottled face, spotted (other than Holsteins), yellow, red-white face, black-white face, red, white, yellow-white face, black, black-mottle face, brown, brindle, and gray, with differences among some colors ( $P < 0.05$ ). At processing, previous environment and pen behavior of other animals cannot be segregated, so it is necessary to view large numbers of animals from different environments to establish correlations. Correlations among breed-types, pen weights, and dressing percentages demonstrated a highly negative correlation ( $r = -0.71$ ;  $P < 0.05$ ) between dressing percentage and balking. Factors decreasing dressing percentage include gut fill, degree of muscling and finish, and weight of the hide, head, and feet. Data, excluding the Holstein breed effect, illustrated negative correlations between pen weight ( $r = -0.49$ ;  $P < 0.05$ ), dressing percentage ( $r = -0.58$ ;  $P < 0.05$ ), and balking. Balking behavior may be related to breed-type effects and negative carcass implications. Balk score associations with accepted temperament indicator tests and individual carcass data are underway to further evaluate these data.

## Introduction

Handling of cattle throughout their lifetime has an impact on learning. Despite their innate gregarious behavior, some learn to avoid places and people which may lead to resistance in moving forward in the working facility. This balking behavior requires stimulus from the handler to coerce the animal and may present a challenge. When standard stimuli fail, animal welfare issues emerge as more persuasive handling aids such as the electric prod are needed to keep animals moving. Electric prod use in 25% of the animals results in an unacceptable rating in virtually all animal welfare audits.

Economically, the time required for handlers to keep cattle moving forward is a loss. The risk of decline in the quality of carcass traits has been correlated to poor temperament and stress before harvest (King et al., 2006; Grandin, 1993). Animals that continually must be coerced may present an increased risk of an unfavorable product.

Coat color and markings may be an indicator of breed-type classification which is confounded with crossbreeding. Identifying balking tendencies based on breed-type and gender is important for animal wellbeing and industry economics. Objectives were to discover if balking behavior has a breed-type predominance, differs between gender, or affects carcass economics.

## Materials and Methods

Subjective observations ( $n = 2375$ ) were taken at a large-capacity beef processing plant in Texas. Animal handlers were trained based on the American Meat Institute Animal Care and Handling Guidelines (2012) and the facility was modernly designed to promote continuous flow of cattle and reduce balking. Line speed was 390/hour. Two consistent university observers recorded data.

One observer recorded color and characteristic markings as animals entered the opening to the indoor working facility. The other observer recorded balking behavior just prior to the center-track restrainer using the following developed Balking Score Criteria: 1 = none; willing forward movement; 2 = stops; then proceeds on own; 3 = persuasion needed, shake of paddle/handling aid or manual tap on rump/tail area; 4 = persistent balk, 2+ persuasion efforts needed to continue forward motion or 1 use of electric prod; 5 = intense balk; electric prod 2 ± times required for continued forward motion.

Recordings were taken for two consecutive days during both "A" and "B" (A shift 5:00 AM to 3:00 PM and B shift 3:00 PM to 11:00 PM) shifts under two different handlers. Cattle were received from 10 different feedlots and presented 14 color/marking combinations. Initial data were analyzed with SAS (SAS Inst., Inc., Cary, N.C.) using PROC MIXED, PROC CORR and PROC FREQ. For PROC MIXED, the model contained fixed effects for feedlot, pen, color, date, sex, and time. Random effects were time within date. Least squares means were used for color and sex with differences significant at  $P < 0.05$ . A separate analysis with PROC CORR excluding Holstein cattle was completed to illustrate beef-type only breed effects.

## Results and Discussion

A single mixed pen containing both steers and heifers displayed a higher ( $P < 0.05$ ) mean balk score than those pens with a single gender. Balking behavior means were similar ( $P > 0.05$ ) between steers and heifers (Table 1).

Balk score means ordered from highest to lowest by color: Holstein, red-mottle face, spotted, yellow, red-white face, black-white face, red, white, yellow-white face, black, black-mottle face, brown, brindle, and gray (Table 2). Holstein and red mottle-face cattle balked

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similarly ( $P > 0.05$ ). Red-mottle face, spotted, and yellow barked similarly ( $P > 0.05$ ). Spotted, yellow, red-white face, black-white face, red, white, yellow-white face, black, black-mottle face, brown, brindle, and gray all barked similarly ( $P > 0.05$ ) and significantly less ( $P < 0.05$ ) than Holstein cattle. Number of animals per color varied. Extremes of the balking score criteria ranked coat colors on a percentage basis. On a percentage basis, Table 3 illustrates the top 5 colors that moved through the processing line most readily were gray, brown, yellow-white face, black, and white. The top 5 colors that gave handlers the most difficulty in maintaining line speed were red-mottle face, Holstein, yellow, spotted, and brown.

Dressing percentage was calculated from average hot carcass weight divided by average live pen weight. Dressing percentage can be affected by gut fill, degree of muscling, degree of fat, and weight of head, feet, and hide. Holsteins, being dairy cattle, generally have larger gut fill and are more lightly muscled than beef cattle. Removing the Holstein data still illustrated a negative relationship with balking behavior, pen weight ( $r = -0.49$ ;  $P < 0.05$ ) and dressing percentage ( $r = -0.58$ ;  $P < 0.05$ ). While correlations do not imply causation, these findings were the foundation to further studies and analyses.

## Implications

Balking behavior shows a breed-type relationship based on coat color. Heifers and steers balk similarly in segregated pens but balk more when comingled. Dressing percentage is negatively related to balking behavior and therefore carcass economics may be impacted.

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**Table 1. Balking score<sup>†</sup> means by sex.**

Sex	Balking Score	Standard Error
Mixed pen (n = 63)	2.4 <sup>a</sup>	0.20
Steers (n = 1341)	2.0 <sup>b</sup>	0.09
Heifers (n = 971)	2.0 <sup>b</sup>	0.09

<sup>†</sup> Balking score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balking requiring 2+ efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

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

**Table 2. Balking score<sup>†</sup> means order from highest to lowest, and percentage represented.**

Color	Color		Balking Score Least		
	Abbreviation	Number	Squares means	Percentage (%)	Standard Error
Holstein	HOLS	322	2.75 <sup>a</sup>	13.7	0.14
Red-mottle face	RMF	15	2.73 <sup>ab</sup>	0.6	0.34
Spotted	SPOT	32	2.16 <sup>bc</sup>	1.4	0.24
Yellow	Y	121	2.09 <sup>bc</sup>	5.1	0.14
Red-white face	RWF	84	2.01 <sup>c</sup>	3.6	0.16
Black-white face	BWF	93	1.88 <sup>c</sup>	3.9	0.15
Red	R	184	1.85 <sup>c</sup>	7.8	0.11
White	W	211	1.82 <sup>c</sup>	8.9	0.11
Black	B	1051	1.81 <sup>c</sup>	44.6	0.08
Black-mottle face	BMF	65	1.81 <sup>c</sup>	2.8	0.18
Brown	BRN	49	1.80 <sup>c</sup>	2.1	0.20
Brindle	BRIN	50	1.79 <sup>c</sup>	2.1	0.19
Total		2358			

<sup>†</sup> Balking score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balking requiring 2+ efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

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

**Table 3. Order of colors by balk scores<sup>†</sup> 1, 4 and 5 by percentage.**

Color	Color Abbreviation	Balk score 1		Balk score 4		Balk score 5	
		Color	%	Color	%	Color	%
Black	B	G	78.0	RMF	46.7	HOL	9.0
Black-mottled face	BMF	BRN	75.5	HOL	37.9	RMF	6.7
Black-white face	BWF	YWF	74.2	SPOT	28.1	Y	5.0
Brindle	BRIN	B	73.7	Y	23.1	RWF	3.6
Brown	BRN	W	73.5	BRN	22.4	B	2.3
Gray	G	R	69.0	YWF	19.3	BWF	2.2
Holstein	HOLS	BWF	68.8	BRIN	18.0	G	2.0
Red	R	BRIN	68.0	W	17.5	W	1.9
Red-mottled face	RMF	BMF	67.7	B	16.3	R	1.6
Red-white face	RWF	Y	63.6	R	16.3	BMF	1.5
Spotted	SPOT	RWF	59.5	G	16.0	BRIN	< 1.0
White	W	SPOT	56.3	BMF	15.3	BRN	< 1.0
Yellow	Y	RMF	46.7	BWF	15.1	YWF	< 1.0
Yellow-white face	YWF	HOL	45.0	RWF	13.1	SPOT	< 1.0

<sup>†</sup> Balk score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2+ efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

**Table 4. Pearson correlation coefficients (r) for all animals and with Holstein cattle data excluded.**

Balk Score <sup>‡</sup>		Pen Weight (lbs),	Dressing Percentage <sup>†</sup> ,
		<i>P</i> -value	<i>P</i> -value
	All animals	0.08 ( <i>P</i> > 0.05)	-0.71 ( <i>P</i> < 0.05)
	Less Holsteins	-0.49 ( <i>P</i> < 0.05)	-0.58 ( <i>P</i> < 0.05)

<sup>†</sup> Dressing percentage: Hot carcass weight/live weight.

<sup>‡</sup> Balk score: 1-5; 1 = no balking, 2 = stops, proceeds on own, 3 = persuasion needed, 4 = persistent balk requiring 2+ efforts to coerce or 1 use of electric prod, 5 = intense balking requiring multiple efforts or 2+ electric prods.

# Population and price differences for Arkansas sale barn marketed calves from 2000 to 2010 due to management influenced phenotype

T.R. Troxel<sup>1</sup> and M.S. Gadberry<sup>1</sup>

## Story in Brief

The objectives of this study were to determine how declining calf supplies from 2000 to 2010 impacted the selling price of calves. Data from weekly Arkansas livestock auctions were collected from January 1 to December 31 in 2000, 2005 and 2010. Data included selling in groups, fill, body condition, and health. Mean selling price for 2000, 2005 and 2010 was \$93.94 ± 12.80, \$117.00 ± 13.41 and \$109.12 ± 13.42 ( $\bar{X} \pm SD$ ; U.S. dollars/cwt.), respectively. Individual price observations were standardized within year ( $\bar{X} = 0$ ). The proportion of calves sold as singles decreased from 82.4% (2000) to 74.8% (2010;  $P < 0.001$ ). The discount for calves sold as singles was greater in 2000 (-\$0.86) compared to 2010 (-\$0.42,  $P < 0.01$ ). The discount for full and tanked calves was similar in 2005 and 2010 but greater than the discount in 2000 ( $P < 0.01$ ). Calves in very thin condition were discounted the greatest (-\$8.66) in 2010, and fleshy calves were discounted the greatest in 2005 (-\$5.78,  $P < 0.01$ ). The discount for fat calves did not differ between 2005 (-\$17.87) and 2010 (-\$12.38) but both were greater than the discount of 2000 (-\$5.67,  $P < 0.01$ ). Most calves were identified as healthy among years (>95%). The discount for sick calves did not differ ( $P > 0.1$ ) among years. Calves exhibiting dead hair coat or stale appearance were discounted similarly in 2005 and 2010 which were greater than those of 2000 ( $P < 0.001$ ). Preconditioned cattle received a greater premium in 2010 (\$6.84) compared to 2005 (\$4.68,  $P < 0.01$ ). The results indicated buyers discounted undesirable management characteristics and were willing to spend more for preconditioned cattle during a period of declining calf supplies.

## Introduction

Sale barns or auction markets are the most preferred method of marketing calves. Across the United States, when cattle or calves permanently left their operations, 90.0% of operations merchandized cattle via the sale barn or auction market. For herd sizes of 1 to 49 and 50 to 99 cows, the percentages of operations sending cattle through a sale barn or auction market was 90.3% and 93.2%, respectively, but that percentage decreased as the herd size increased (USDA, 2009).

Cow-calf producers are challenged to produce calves that are acceptable to the industry. Reports indicated that breed or breed type, health, sex, frame and muscle scores, age and source verification, and other factors affect calf selling price (Troxelet al., 2002; Barham and Troxel 2007; and Troxel and Barham, 2012).

The U.S. and Arkansas calf crop decreased 8.2% (3.2 million cattle) and 4.8% (40 thousand cattle) from 2000 to 2010, respectively (USDA/NASS, 2012). The objectives of this study were to determine whether the management-influenced phenotypic factors affecting the selling price of feeder calves changed from 2000, 2005 to 2010 and to examine the perception that discounts narrow or even disappear as calf supplies decrease.

## Materials and Methods

Five USDA certified livestock market reporters collected data from weekly livestock auctions in Arkansas from January 1 to December 31 in 2000, 2005 and 2010. Market reporters collected information from the same 12 markets in 2000, 2005 and 2010. Livestock auctions surveyed were located in Ash Flat, Charlotte, Conway, Fort Smith, Glenwood, Green Forest, Hope, Ola, Ozark, Pochontas, Ratcliff and Springdale. Data collected included group size (singles, 2 to 5 calves, or ≥ 6 calves), fill (gaunt, shrunk, average, full, or tanked), condition (very thin, thin, average, fleshy, or fat),

and health (dead hair, stale, morbid, bad eye(s), lame, healthy, or preconditioned). A total of 382,446; 482,238 and 475,279 calves were sold through these livestock auctions in 2000, 2005 and 2010, respectively. Data were randomly collected (every 6th to 7th calf) on 48,463 (12.7%), 84,749 (17.6%) and 56,968 (12.0%) calves in 2000, 2005 and 2010, respectively.

The percentage of calves within group size, fill, condition, and health were determined by the frequency procedure of SAS (SAS Inst. Inc., Cary, N.C.) based on the number of lots sold. Chi-squared analysis of SAS was used to test for significant changes in the proportion of cattle representative of each descriptive characteristic among years. Cattle marketed in groups were excluded from the analysis of individual calf descriptive characteristics influences on selling prices. Individual price observations were standardized within year ( $\bar{X} = 0$ ) and the standardized price was the dependent variable. Calf characteristics were analyzed individually as independent variables in which the model included month and body weight as covariates. All other variables contributed to the error sum of squares. The ANOVA was performed with the GLM procedure of SAS. Least-squares means ( $\pm SE$ ) were generated, separated based on predicted differences, and both are reported throughout. All selling prices reported are in U.S. dollars/cwt.

## Results and Discussions

The mean selling prices for 2000, 2005 and 2010 were \$93.94 ± 12.80, \$117.00 ± 13.41, and \$109.12 ± 13.42/cwt. ( $\bar{X} \pm SD$ ), respectively (Fig. 1). Each year showed a typical seasonal price trend, with the higher prices occurring in the spring and the lower prices occurring in the late summer and early fall. Seasonal price patterns for calves are relatively consistent and associated with production patterns.

**Group Size.** The percentage of calves sold as singles decreased from 2000 to 2010 ( $P < 0.01$ ; Table 1). Arkansas cow-calf producers

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increased the percentage sold in groups of 2 to 5 calves from 14.0% to 19.7% and in groups of  $\geq 6$  calves from 3.6% to 5.5% in 2000 and 2010, respectively ( $P < 0.01$ ). The selling prices for calves sold individually were below the average for 2000 and 2010 but not in 2005 and all prices differed ( $-\$0.86 \pm 0.05$ ,  $\$0.04 \pm 0.06$  and  $-\$0.42 \pm 0.07$ ;  $\bar{X} \pm SE$ ;  $P < 0.01$ ). Prices for calves sold in groups were higher in 2005 and 2010 than in 2000 ( $P < 0.01$ ). The selling prices for those sold in groups (2 to 5 calves or  $\geq 6$  calves) in 2005 and 2010 were not different ( $P > 0.10$ ).

*Fill.* The increase in the frequency of gaunt calves in 2005 was probably related to the drought conditions in much of Arkansas ( $P < 0.01$ ; Table 2). Arkansas experienced the sixth-driest year on record in 2005 (National Weather Service-Little Rock, 2012). The percentage of shrunk calves increased over time; whereas, the percentage of full and average fill calves decreased over time ( $P < 0.01$ ). Even with lesser feeder calf supplies in 2005 and 2010, full and tanked (excessively full) feeder calves received greater discounts than in 2000 ( $P < 0.01$ ).

Gaunt calves received their greatest premiums in 2010 ( $P < 0.01$ ) compared to 2000 and 2005. Average fill calves received discounts all 3 years. Buyers found gaunt and shrunk feeder cattle as desirable but were unwilling to absorb the shrink on full and tanked feeder calves and did not pay average market price for those calves regardless of the change of the supply of cattle from 2000 to 2010.

*Body Condition.* As with body fill, calf body condition was probably affected by the severe drought conditions in 2005, resulting in an increase in the frequency of very thin calves compared with 2000 and 2010 ( $P < 0.01$ ; Table 3). The percentage of calves with a thin or average body condition was greater in 2010 than 2000; whereas, the percentage of calves with a fleshy or fat body condition was less in 2010 than 2000 ( $P < 0.01$ ). In 2000 and 2010, very thin calves were discounted but in 2005 they received a premium and all means differed from one another ( $P < 0.01$ ). Thin calves received premiums in 2000 and 2010 and received a slight discount in 2005 ( $P < 0.01$ ). The discounts for fleshy calves were greater in 2005 ( $P < 0.01$ ) than for 2000 and 2010. Fat cattle were discounted all 3 years with the greatest discounts recorded in 2005 and 2010. Thin calves offer an opportunity for buyers to increase profits through compensatory gain. Fleshy or fat calves are discounted because their gains are compromised during the early phases of backgrounding. The cow-calf producer absorbed the extra feed cost that resulted in the extra condition and then was discounted when the calf was sold. Feeder calves of average body condition received a slightly higher selling price in 2005 compared with 2000 and 2010 ( $P < 0.01$ ). Buyers continued to discount fleshy and fat feeder cattle even though cattle supplies declined from 2000 to 2010; whereas, the selling prices of feeder cattle classified as very thin, thin and average was inconsistent.

*Health.* The percentage of calves with a poor health condition [dead hair, stale (dull or lifeless behavior), morbid, bad eye(s), and lame] was low in 2000 and even lower in 2005 and 2010 (2.1% vs. 1.1% and 0.9%;  $P < 0.01$ ; Table 4). When feeder calves were obviously morbid at the livestock auction, buyers severely discounted the unhealthy calves. The discounts for feeder calves that were morbid or had bad eyes were the same from 2000 to 2010 ( $P > 0.10$ ). Discounts were greater in 2005 and 2010 for calves that were lame,

stale or had dead hair compared with 2000. When compared among years, healthy calves were valued less in 2000 but received premiums in 2005 and 2010 and all prices differed ( $P > 0.01$ ).

Preconditioning is designed to ensure the calf is bunk broke, reduce incidence of bovine respiratory disease by increasing the immunity of the calf in preparation for the stress of weaning and shipping, and recover from the stress of maternal separation. Data were not collected on preconditioned calves in 2000 but were collected in 2005 and 2010. Only normal sales were recorded; no special preconditioned sales are represented in the data set. Calves that were announced as preconditioned at the time of sale are included in this data set. Approximately 3.6% of the calves sold were announced as being preconditioned. Premium received for preconditioned calves was greater in 2010 than in 2005 ( $P < 0.01$ ). With only 2 years reported (2005 and 2010), buyers appear to see the value of preconditioned calves and are paying more for them even if cattle aren't part of a special precondition calf sale.

## Implications

Market conditions determine the base price for feeder calves, and adjustments to the final sale price are made based on perceived profit potential. As a general rule, when calf supplies are short and prices are high, buyers may increase discounts certain characteristics (selling as singles, full and tanked calves, fleshy and fat calves, small framed and light muscled calves, etc.) because of the greater investment risk in a high price, calf market. In other words, buyers are spending more money to purchase calves for the same amount of management risk (such as health and weight gain expectations); therefore they "hedge" their risk by discounting cattle where they can.

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**Table 1. Group size frequency distribution and the 2000, 2005 and 2010 Arkansas selling price reported as deviations from the respective means.**

Group size	Frequency percentage <sup>†</sup>			Deviation from the respective means <sup>‡,§</sup>		
	2000	2005	2010	2000	2005	2010
1	82.4	77.2	74.8	-\$0.86 ± 0.05 <sup>c</sup>	\$0.04 ± 0.06 <sup>a</sup>	-\$0.42 ± 0.07 <sup>b</sup>
2 to 5 calves	14.0	17.7	19.7	-\$0.10 ± 0.11 <sup>b</sup>	\$2.29 ± 0.12 <sup>a</sup>	\$2.34 ± 0.13 <sup>a</sup>
≥ 6 calves	3.6	5.1	5.5	\$1.24 ± 0.23 <sup>b</sup>	\$4.39 ± 0.23 <sup>a</sup>	\$4.94 ± 0.25 <sup>a</sup>

<sup>†</sup> The frequency percentage of calves sold as singles decreased from 2000 to 2010 ( $P < 0.01$ ).

<sup>‡</sup> Mean selling price for 2000, 2005 and 2010 was \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>§</sup> LS means ± SE.

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).

**Table 2. Body fill frequency distribution and the 2000, 2005 and 2010 Arkansas selling price reported as deviations from the respective means.**

Body fill	Frequency percentage			Deviation from the respective means <sup>†,‡</sup>		
	2000	2005	2010	2000	2005	2010
Gaunt	18.5	25.0	18.8	\$2.63 ± 0.10 <sup>b</sup>	\$2.21 ± 0.10 <sup>c</sup>	\$5.44 ± 0.13 <sup>a</sup>
Shrunk <sup>§</sup>	20.6	22.0	33.5	\$1.49 ± 0.09 <sup>b</sup>	\$2.90 ± 0.11 <sup>a</sup>	\$1.02 ± 0.10 <sup>c</sup>
Average <sup>¶</sup>	51.1	50.4	44.8	-\$1.62 ± 0.06 <sup>b</sup>	-\$0.74 ± 0.07 <sup>a</sup>	-\$1.64 ± 0.09 <sup>b</sup>
Full <sup>¶</sup>	9.5	2.5	2.9	-\$6.17 ± 0.14 <sup>a</sup>	-\$7.46 ± 0.32 <sup>b</sup>	-\$7.42 ± 0.34 <sup>b</sup>
Tanked <sup>#</sup>	0.3	0.1	0.1	-\$8.66 ± 0.84 <sup>a</sup>	-\$20.37 ± 2.29 <sup>b</sup>	-\$17.63 ± 1.98 <sup>b</sup>

<sup>†</sup> Mean selling price for 2000, 2005 and 2010 was \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>‡</sup> LS means ± SE.

<sup>§</sup> Percentage of shrunk calves increased over time ( $P < 0.01$ ).

<sup>¶</sup> Percentage of full and average fill calves decreased over time ( $P < 0.01$ ).

<sup>#</sup> Excessively full.

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).

**Table 3. Body condition frequency distribution and the 2000, 2005 and 2010 Arkansas selling price reported as deviations from the respective means.**

Body condition	Frequency percentage			Deviation from the respective means <sup>†,‡</sup>		
	2000	2005	2010	2000	2005	2010
Very thin <sup>§</sup>	1.7	25.4	0.7	-\$5.98 ± 0.33 <sup>b</sup>	\$2.20 ± 0.10 <sup>a</sup>	-\$8.66 ± 0.74 <sup>c</sup>
Thin <sup>¶</sup>	25.7	10.1	33.0	\$2.20 ± 0.08 <sup>a</sup>	-\$0.37 ± 0.16 <sup>c</sup>	\$1.76 ± 0.10 <sup>b</sup>
Average <sup>¶</sup>	59.0	61.9	62.0	-\$1.07 ± 0.06 <sup>c</sup>	\$0.44 ± 0.07 <sup>a</sup>	\$0.10 ± 0.08 <sup>b</sup>
Fleshy <sup>#</sup>	12.8	2.6	4.3	-\$3.41 ± 0.12 <sup>a</sup>	-\$5.78 ± 0.32 <sup>b</sup>	-\$4.11 ± 0.29 <sup>a</sup>
Fat <sup>#</sup>	0.8	< 0.1	< 0.1	-\$5.67 ± 0.47 <sup>a</sup>	-\$17.87 ± 2.51 <sup>b</sup>	-\$12.38 ± 2.59 <sup>b</sup>

<sup>†</sup> Mean selling price for 2000, 2005 and 2010 were \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>‡</sup> LS means ± SE.

<sup>§</sup> The percentage of very thin body condition calves was greater in 2005 than in 2000 and 2010 ( $P < 0.01$ ).

<sup>¶</sup> The percentage of thin and average body condition calves was greater in 2010 than in 2000 ( $P < 0.01$ ).

<sup>#</sup> The percentage of fleshy and fat body condition calves was lesser in 2005 and 2010 than in 2000 ( $P < 0.01$ ).

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).

**Table 4. Health status frequency distribution and the 2000, 2005 and 2010 Arkansas selling price reported as deviations from the respective means.**

Health status	Frequency percentage			Deviation from the respective means <sup>†,‡</sup>		
	2000	2005	2010	2000	2005	2010
Dead hair	0.2	0.2	0.2	-\$15.02 ± 1.15 <sup>a</sup>	-\$23.40 ± 1.27 <sup>b</sup>	-\$21.97 ± 2.01 <sup>b</sup>
Stale <sup>§</sup>	1.4	0.3	0.2	-\$15.48 ± 1.01 <sup>a</sup>	-\$26.16 ± 1.91 <sup>b</sup>	-\$32.61 ± 4.76 <sup>b</sup>
Morbid	0.2	0.1	< 0.1	-\$9.96 ± 0.87 <sup>a</sup>	-\$9.13 ± 1.33 <sup>a</sup>	-\$8.95 ± 1.23 <sup>a</sup>
Bad eye (s)	0.2	0.3	0.3	-\$11.19 ± 0.98 <sup>a</sup>	-\$11.82 ± 1.00 <sup>a</sup>	-\$12.04 ± 1.10 <sup>a</sup>
Lame	0.1	0.2	0.1	-\$10.58 ± 0.37 <sup>a</sup>	-\$15.77 ± 0.88 <sup>b</sup>	-\$17.97 ± 1.32 <sup>b,c</sup>
Healthy	97.9	95.4	95.6	-\$0.44 ± 0.04 <sup>a</sup>	\$0.69 ± 0.05 <sup>b</sup>	\$0.33 ± 0.06 <sup>c</sup>
Preconditione <sup>d¶</sup>	NA	3.7	3.6	NA	\$4.68 ± 0.27 <sup>a</sup>	\$6.84 ± 0.31 <sup>b</sup>

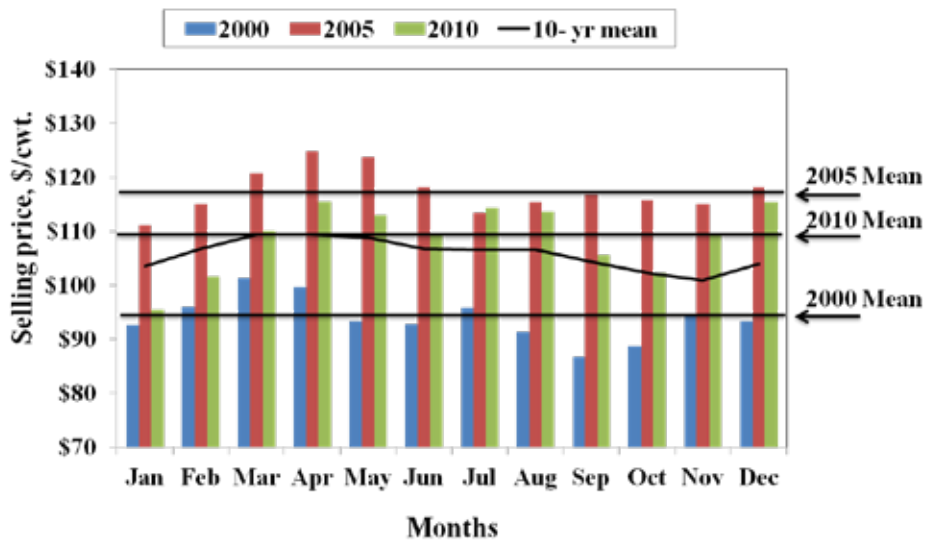
<sup>†</sup> Mean selling price for 2000, 2005 and 2010 were \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>‡</sup> LS means ± SE.

<sup>§</sup> Dull or lifeless behavior.

<sup>¶</sup> Data was not collected on preconditioned calves in 2000.

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).



<sup>a</sup> The 10 yr monthly mean represents 500 to 600 lb steers for 2000 to 2010.

**Fig. 1. The 10 year monthly mean<sup>a</sup> and the 2000, 2005 and 2010 monthly and yearly mean selling prices for feeder cattle sold in Arkansas livestock auctions (Cheney, 2011).**

# Population and price differences for Arkansas sale barn marketed calves from 2000 to 2010 due to genetically influenced phenotype

T.R. Troxel<sup>1</sup> and M.S. Gadberry<sup>1</sup>

## Story in Brief

The objectives of this study were to determine how declining calf supplies from 2000 to 2010 impacted the selling price of calves due to genetic influences. Data from weekly Arkansas livestock auctions were collected from January 1 to December 31 in 2000, 2005 and 2010. Data included gender, breed or breed type, color, muscle thickness, horn status, frame score, and body weight. Mean selling price for 2000, 2005 and 2010 was \$93.94 ± 12.80, \$117.00 ± 13.41 and \$109.12 ± 13.42 (mean ± SD; U.S. dollars/cwt.), respectively. Steers received the greatest premium in 2010 (\$8.21 ± 0.09) compared to 2000 (\$5.18 ± 0.07) and 2005 (\$6.00 ± 0.07;  $P < 0.01$ ); whereas, bulls and heifers received greater discounts from 2000 to 2010. Of the breeds evaluated, only Angus × Brahman received increased premiums in 2000 (\$0.55 ± 0.13), 2005 (\$1.47 ± 0.13) and 2010 (\$3.03 ± 0.19;  $P < 0.01$ ). The selling price of black-white face, yellow and yellow-white face calves was above the average and the selling price of gray-white face, red, red-white face and spotted or striped calves was below the average in all 3 years. Horned calves received greater discounts in 2010 (-\$4.25 ± 0.20) than in 2000 (-\$1.17 ± 0.09;  $P < 0.01$ ). Large- and medium-framed calves were discounted in 2000 but received premiums in 2005 and 2010 ( $P < 0.01$ ). Calves with a number 1 muscle score received a premium (\$0.51 ± 0.04, \$2.75 ± 0.06 and \$2.21 ± 0.06 for 2000, 2005 and 2010, respectively;  $P < 0.01$ ). Small-framed calves and calves classified muscle score 2 or 3 were discounted all 3 years. The same factors affecting the selling price of calves sold through Arkansas livestock auction in 2000 continued to affect the selling price in 2010 and in some cases discounts were greater even with a declining U.S. cattle inventory.

## Introduction

Sale barns or auction markets remain the method by which most producers market calves. Across the United States, when cattle or calves permanently left their operations, 90.0% of operations merchandized cattle via the sale barn or auction market. For herd sizes of 1 to 49 and 50 to 99 cows, the percentages of operations sending cattle through a sale barn or auction market was 90.3% and 93.2%, respectively, but that percentage decreased as the herd size increased (USDA, 2009). Cow-calf producers are challenged to produce calves that are acceptable to the industry. Reports indicated that breed or breed type, health, sex, frame and muscle scores, age and source verification, and other factors affect calf selling price (Troxel et al., 2002; Barham and Troxel 2007; and Troxel and Barham, 2012). The U.S. and Arkansas calf crop decreased 8.2% (3.2 million cattle) and 4.8% (40 thousand cattle) from 2000 to 2010, respectively (USDA/NASS, 2012). The objectives of this study were to determine whether the genetically influenced phenotypic factors affecting the selling price of feeder calves changed from 2000, 2005 to 2010 and to examine the perception that discounts narrow or even disappear as calf supplies decrease.

## Material and Methods

Five USDA certified livestock market reporters collected data from weekly livestock auctions in Arkansas from January 1 to December 31 in 2000, 2005 and 2010. Market reporters collected information from the same 12 markets in 2000, 2005 and 2010. Livestock auctions surveyed were located in Ash Flat, Charlotte, Conway, Fort Smith, Glenwood, Green Forest, Hope, Ola, Ozark, Pochahontas, Ratchiff and Springdale. Data collected included calf gender (bull, steer, or heifer), breed or breed type, color (black, black-white face, gray, gray-white face, red, red-white face, spotted

or striped, white, yellow or yellow-white face), muscle thickness (1 = moderately thick throughout, 2, 3, or 4 = least amount of muscle thickness), horn status (polled or horned), frame score (large, medium, or small), and body weight. A total of 382,446; 482,238; and 475,279 calves were sold through these livestock auctions in 2000, 2005 and 2010, respectively. Data were randomly collected (every 6th to 7th calf) on 48,463 (12.7%), 84,749 (17.6%) and 56,968 (12.0%) calves in 2000, 2005 and 2010, respectively.

Frame and muscle scores were determined based on the U.S. Standards for Grades of Feeder Cattle (USDA, 2000). On October 1, 2000, USDA changed the muscle scoring system for estimating muscle thickness (USDA, 2000). Therefore to compare the impact of muscle scores on selling price only data collected on and after October 1 were compared.

*Statistical Analysis.* The percentage of calves within gender, breed or breed type, color, horn status, frame score, muscle score and body weight group were determined by the frequency procedure of SAS (SAS Inst. Inc., Cary, N.C.) based on the number of lots sold. Chi-squared analysis of SAS was used to test for significant changes in the proportion of cattle representative of each descriptive characteristic among years. Cattle marketed in groups were excluded from the analysis of individual calf descriptive characteristics influences on selling prices. Individual price observations were standardized within year (mean = 0) and the standardized price was the dependent variable. Calf characteristics were analyzed individually as independent variables in which the model included month and body weight as covariates. All other variables contributed to the error sum of squares. The ANOVA was performed with the GLM procedure of SAS. Least-squares means (± SE) were generated, separated based on predicted differences, and both are reported throughout. Because all colors are not represented within each breed or breed type, color and breed or breed type data are somewhat inherently confounded. All selling prices reported are in U.S. dollars/cwt.

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## Results and Discussion

The mean selling prices for 2000, 2005 and 2010 were \$93.94  $\pm$  12.80, \$117.00  $\pm$  13.41, and \$109.12  $\pm$  13.42/cwt. ( $\bar{X}$   $\pm$  SD), respectively. The percentage of calves in the 300 to 349 lbs and 350 to 399 lbs body weight groups decreased from 2000 to 2010 ( $P < 0.01$ ; Fig. 1) and the percentage of calves in the 550 to 559 lbs and 600 to 649 lbs body weight groups increased from 2000 to 2010 ( $P < 0.01$ ). This was attributed to either improved beef cattle genetics or a change in management. The price of corn steadily increased during the marketing period of 2000 to 2010 which generally increases the value of calf gains from pasture. Arkansas cattle producers may have retained ownership post weaning in order to capture additional profits.

**Gender.** The percentage male calves sold as steers differed among years ( $P < 0.01$ ) with a low of 31.5% in 2000, a high of 39.9% in 2005, and 2010 intermediate (33.9%; Table 1). Steers received the greatest premium (\$8.21  $\pm$  0.09) in 2010 compared to 2000 (\$5.18  $\pm$  0.07) and 2005 (\$6.00  $\pm$  0.07;  $P < 0.01$ ). The selling price of bulls was \$0.30  $\pm$  0.08, -\$0.10  $\pm$  0.13 and \$1.38  $\pm$  0.12 in 2000, 2005 and 2010, respectively, and differed among years ( $P < 0.01$ ). Although the selling price of bulls was highest in 2010, so was the discount compared to steers. In 2000, 2005 and 2010, bulls were discounted compared to steers by \$4.88, \$6.10 and \$6.83, respectively. Reflective of discounted selling prices for bull calves, stocker operators and feedyards emphasize the need to castrate males before selling (Troxel et al., 2002). Even with declining calf numbers, market signals continue to favor the castration of bull calves. Heifers were discounted more in 2010 (-\$5.79  $\pm$  0.08) than in 2000 and 2005 (-\$5.27  $\pm$  0.06 and -\$3.62  $\pm$  0.07;  $P < 0.01$ ). In this study, heifers were discounted \$10.45, \$9.62 and \$14.00 in 2000, 2005 and 2010, respectively, compared to steers.

**Breed or Breed Type.** Livestock market reporters evaluated each feeder calf and determined its breed or breed type based on frame score, muscle thickness, color, breed characteristics, and body structure. Therefore, breeds or breed types were based on reporter perception rather than by actual known composition. Thirty breeds or breed types were identified in 2000, 2005 and 2010 (Table 2). The frequency percentage of Angus, Angus  $\times$  Charolais and Angus  $\times$  Hereford calves increased ( $P < 0.01$ ) and the frequency percentage of Hereford  $\times$  Limousin, Limousin, and Simmental calves decreased ( $P < 0.01$ ) from 2000 to 2010.

Only Angus  $\times$  Brahman received year-by-year increase premiums (\$0.55  $\pm$  0.13, \$1.47  $\pm$  0.13 and \$3.03  $\pm$  0.19 in 2000, 2005 and 2010, respectively;  $P < 0.01$ ) while Saler (-\$4.63  $\pm$  0.45, -\$6.84  $\pm$  0.69 and -\$10.61  $\pm$  0.85 in 2000, 2005 and 2010, respectively) and Longhorn (-\$18.39  $\pm$  0.81, -\$25.96  $\pm$  1.05 and -\$35.99  $\pm$  2.33 in 2000, 2005 and 2010, respectively) received year-by-year discounts in 2000, 2005 and 2010 ( $P < 0.01$ ). Angus  $\times$  Limousin, Hereford  $\times$  Charolais, Angus  $\times$  Simmental, Hereford  $\times$  one-fourth Brahman and Hereford  $\times$  Simmental premiums or discounts did not differ ( $P > 0.10$ ) in 2000, 2005 and 2010.

Breed or breed types that received the same premium or discount in 2000 and 2005 but received a lesser price ( $P < 0.01$ ) in 2010 were Charolais, Charolais  $\times$  Limousin, Limousin  $\times$  one-fourth Brahman and Simmental. Hereford cattle were discounted in 2000 (-\$9.79  $\pm$  0.27) and 2005 (-\$9.13  $\pm$  0.42) but received a premium in 2010 (\$1.70  $\pm$  0.16;  $P < 0.01$ ).

The discounts for Angus  $\times$  Charolais  $\times$  Hereford and Charolais  $\times$  Brahman were greater ( $P < 0.01$ ) in 2000 but were reduced for Charolais  $\times$  Brahman in 2005 and 2010 and Angus  $\times$  Charolais  $\times$  Hereford received premiums in 2005 and 2010 but were not

different ( $P > 0.10$ ). Limousin discounts in 2005 (-\$0.48  $\pm$  0.17) and premiums in 2010 (\$0.15  $\pm$  0.22) were not different ( $P > 0.10$ ) but were numerically higher than 2000 (\$0.80  $\pm$  0.12;  $P < 0.01$ ).

Of the 30 breed or breed types identified in this data set, only eight recorded selling prices above the mean for all 3 years and 13 recorded selling prices below the mean for all 3 years (Table 2). The breed or breed types that sold for above the means for the 3 years were Angus  $\times$  Brahman, Angus  $\times$  Hereford, Angus  $\times$  Hereford  $\times$  one-fourth Brahman, Angus  $\times$  Limousin, Charolais, Charolais  $\times$  Limousin, Hereford  $\times$  Brahman  $\times$  Angus and Hereford  $\times$  Charolais. The breed or breed types that sold for below the means for the 3 years were Angus  $\times$  Simmental, Brahman, one-half Brahman cross, one-fourth Brahman cross, Brahman  $\times$  Hereford, Brahman  $\times$  Limousin, Charolais  $\times$  Brahman, Hereford  $\times$  one-fourth Brahman, Hereford  $\times$  Simmental, Limousin  $\times$  one-fourth Brahman, Longhorn, Saler and Simmental.

**Color.** Calf color frequency distribution and the 2000, 2005 and 2010 Arkansas selling prices are reported in Table 3. Black, black-white face, and gray feeder calves were the only colors that increased in frequency from 2000 to 2010 ( $P < 0.01$ ). This can be largely attributed to the increased popularity of black-colored sires. Red, red-white face, white and yellow-white face calves decreased in frequency from 2000 to 2010 ( $P < 0.01$ ). Selling price based on color seemed to be somewhat contradictory. Selling prices for grey-white face, yellow, yellow-white face calves were not different ( $P > 0.10$ ) in 2000 and 2010 but both years were different ( $P < 0.01$ ) when compared to 2005. The selling prices for black and black-white face calves were not different ( $P > 0.10$ ) in 2005 and 2010 but both years were greater than 2000 ( $P < 0.01$ ). Red-white face and spotted or striped calves selling prices were discounted the same in 2000 and 2005 ( $P > 0.01$ ) but the discount was greater in 2010 ( $P < 0.01$ ). Discounts for white and red calves increased from 2000 to 2010 ( $P < 0.01$ ) and the selling price for grey calves differed for each year ( $P < 0.01$ ).

**Horn Status.** Although fewer horned calves were sold (25.1%, 13.1%, and 9.2% for 2000, 2005 and 2010, respectively;  $P < 0.01$ ; Table 4), they received greater discounts over time (-\$1.17  $\pm$  0.09, -\$2.48  $\pm$  0.14 and -\$4.25  $\pm$  0.20 for 2000, 2005 and 2010, respectively;  $P < 0.01$ ). Buyers paid a premium for polled calves in 2005 and 2010 (\$1.15  $\pm$  0.06 and \$0.93  $\pm$  0.06;  $P < 0.01$ ). In 2010 the selling price difference between horned and polled feeder calves was \$5.18. Although feeder calf supplies were smaller in 2010, buyers discounted horned feeder calves in 2005 and 2010 compared to 2000.

**Frame Scores.** The percentage of large-framed calves changed over time ( $P < 0.01$ ) and ranged from 55.9% in 2000 to 65.9% in 2005 (Table 5). The percentage of small-framed calves marketed remained 1% or less throughout the 10-year period. The large-framed calves were discounted in 2000 (-0.84  $\pm$  0.06) but received premiums in 2005 and 2010 (\$0.49  $\pm$  0.06 and \$0.74  $\pm$  0.08, respectively) and the means were differed for all 3 years ( $P < 0.01$ ). The selling price for medium-framed calves was also different for all 3 years ( $P < 0.01$ ) with the greatest premium received in 2005 (-\$0.05  $\pm$  0.07, \$1.32  $\pm$  0.09 and \$0.24  $\pm$  0.10, for 2000, 2005 and 2010, respectively). The difference between the large- and medium-framed selling prices was very small (\$0.79, \$0.83 and \$0.50 for 2000, 2005 and 2010, respectively). In the current study, it is difficult to determine how the reduction of calf inventory and buyer's preferences of large- and medium-framed cattle affected selling price. Since large- and medium-framed cattle are acceptable frame sizes and 99% of the calves fit those frame scores, other characteristics may be more influential on determining price.

Small-framed feeder calves were discounted in all 3 years and the discounts were not different ( $-\$16.54 \pm 0.44$ ,  $-\$17.84 \pm 0.65$  and  $-\$16.42 \pm 0.67$  for 2000, 2005 and 2010, respectively;  $P > 0.10$ ). In this analysis, small-framed calves were discounted \$15.70, \$18.33 and \$17.16 compared to large-framed calves for 2000, 2005 and 2005, respectively. These data demonstrate that feeder calf buyers are sending a clear financial message to the cow-calf industry that small-framed feeder calves are not desirable.

**Muscle Scores.** On October 1, 2000, USDA changed the muscle scoring system for estimating muscle thickness (USDA, 2000). Therefore to compare the impact of muscle scores on selling price only data collected on and after October 1, 2000 were compared to the October thru December 2005 and 2010 muscle scores. The frequency distribution and selling price comparisons by muscle score are reported in Table 6. There were only 9, 5 and 2 calves in the muscle score 4 category for 2000, 2005 and 2010, respectively, therefore, muscle score 4 data are not reported. The percentage of muscle score 1 calves differed among years ( $P < 0.01$ ) with 85.4%, 75.3% and 82.0% in 2000, 2005 and 2010, respectively. Calves with a number 1 muscle score received a premium for all 3 years, but a greater premium was detected in 2005 ( $\$0.51 \pm 0.04$ ,  $\$2.75 \pm 0.06$  and  $\$2.21 \pm 0.06$ , for 2000, 2005 and 2010, respectively;  $P < 0.01$ ). Calves with a muscle score of 2 were discounted, but the discount in 2000 ( $-\$8.49 \pm 0.11$ ) was greater ( $P < 0.01$ ) than the discounts in 2005 ( $-\$5.40 \pm 0.10$ ) and 2010 ( $-\$5.91 \pm 0.13$ ). The discounts for calves with muscle scores of 3 were severe and increased ( $P < 0.01$ ) with year ( $-\$15.93 \pm 1.16$ ,  $-19.37 \pm 0.45$  and  $-\$21.78 \pm 0.59$  in 2000, 2005 and 2010, respectively).

**Weight Group.** Buyers usually pay a higher price per pound for lightweight feeder calves because the cost of adding weight is generally less than the value of gain. The 2000, 2005 and 2010 frequency percentages and selling prices by body weight groups are reported in Fig. 1 and Table 7, respectively. From 2000 to 2010, the percentage of lightweight calves (300 to 349 lbs and 350 to 399 lbs body weight groups) decreased ( $P < 0.01$ ); whereas, the body weight groups of 550 to 549 lbs and 600 to 649 lbs increased ( $P < 0.01$ ). This may be due to increased genetic selection for weaning weight. For the two lightest body weight groups (<300 and 300-349 lbs), the premiums above the mean declined from 2000 to 2010. This may be indications that demand for extremely light calves declined due to increased feed costs resulting in an increase cost of gain. Oftentimes stocker operators do not have the facilities to manage calves of this weight group.

In 2005, the calves in the 350–399 and 400–449 lbs body weight groups received a greater selling price as compared to 2000 and 2010 ( $P < 0.01$ ). The year 2005 was very dry (National Weather Service-Little Rock, 2012) and pastures were stressed, but corn was \$2.00/bu. Due to the low price of off-farm feed resources, producers may have seen an opportunity to purchase these calves and capitalize on relatively inexpensive gains, resulting in the greater price of light weight calves in 2005 compared to 2000 or 2010.

The discounts for the calves in the 450–499, 500–549, 550–599 and 600–649 lbs body weight groups decreased from 2000 to 2010. These weight groups make up approximately 54% of the calves sold. This may be due to increased demand and reduced supplies. The calves in the 650–699, 700–749, 750–799 and >800 lbs weight group in 2005 generally received greater discounts compared to 2000 and 2010. Arkansas predominately consists of cow/calf and stocker operations and does not have a feedlot presence. Perhaps due to the 2005 drought, the pasture cost of gain of the heavier weight cattle was too great and therefore, the demand declined resulting in the discount.

Market conditions determine the base price for feeder calves, and adjustments to the final sale price are made based on perceived profit potential. Animals with greater perceived profit risk received lower prices. A number of factors affecting the selling of feeder calves did not change from 2000 to 2010 even with declining cattle inventory. These included: 1) steers sold for a higher selling price than bulls, and heifers were discounts approximately \$10/cwt., 2) breeds or breed types that sold for a premium among all 3 years were Angus  $\times$  Brahman, Angus  $\times$  Hereford, Angus  $\times$  Hereford  $\times$  one-fourth Brahman, Angus  $\times$  Limousin, Charolais, Charolais  $\times$  Limousin, Hereford  $\times$  Brahman  $\times$  Angus and Hereford  $\times$  Charolais and the breeds or breed types that sold for below the means for the 3 years were Angus  $\times$  Simmental, Brahman, one-half Brahman cross, one-fourth Brahman cross, Brahman  $\times$  Hereford, Brahman  $\times$  Limousin, Charolais  $\times$  Brahman, Hereford  $\times$  one-fourth Brahman, Hereford  $\times$  Simmental, Limousin  $\times$  one-fourth Brahman, Longhorn, Saler and Simmental, 3) the selling prices of black-white face, yellow and yellow-face calves were consistently above average; whereas, the selling prices of gray-white face, red, red-white face and spotted or striped calves were consistently below average, 4) polled cattle remained more desirable, 5) small-framed cattle continued to be heavily discounted, 6) muscle score 1 cattle were priced consistently above average; whereas, buyers discounted calves with muscle scores 2 and 3s.

## Implications

Genetic factors that improved calf value included 1) breed characteristics of Angus  $\times$  Brahman, Angus  $\times$  Hereford, Angus  $\times$  Hereford  $\times$  one-fourth Brahman, Angus  $\times$  Limousin, Charolais, Charolais  $\times$  Limousin, Hereford  $\times$  Brahman  $\times$  Angus or Hereford  $\times$  Charolais; 2) coat color associated with black-white face, yellow or yellow-face; 3) polled or dehorned; 4) large- or medium framed and 5) muscle score 1. If cattle are known to be discounted at local sale barns, producers can elect to market the cattle via different means (directly to feedlots, grade and yield, etc.). This analysis can assist producer in making marketing decisions.

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**Table 1. Calf gender frequency distribution and the 2000, 2005 and 2010 Arkansas selling price reported as deviations from the respective means.**

Calf gender	Frequency percentage <sup>†</sup>			Deviation from the respective means <sup>†, §</sup>		
	2000	2005	2010	2000	2005	2010
Steers	31.5	39.9	33.9	\$5.18 ± 0.07 <sup>a</sup>	\$6.00 ± 0.07 <sup>b</sup>	\$8.21 ± 0.09 <sup>c</sup>
Bulls	23.2	12.9	20.5	\$0.30 ± 0.08 <sup>a</sup>	-\$0.10 ± 0.13 <sup>b</sup>	\$1.38 ± 0.12 <sup>c</sup>
Heifers	45.3	47.2	45.6	-\$5.27 ± 0.06 <sup>a</sup>	-\$3.62 ± 0.07 <sup>b</sup>	-\$5.79 ± 0.08 <sup>c</sup>

<sup>†</sup> The frequency percentage of steer, bull and heifer calves differ among years ( $P < 0.01$ ).

<sup>‡</sup> Mean selling price for 2000, 2005 and 2010 was \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>§</sup> LS means ± SE.

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).

**Table 2. Breed or breed type frequency distribution and the 2000, 2005 and 2010 Arkansas selling price reported as deviations from the respective means.**

Breed or breed types	Frequency percentage			Deviation from the respective means <sup>†, ‡</sup>		
	2000	2005	2010	2000	2005	2010
Angus <sup>§</sup>	7.0	10.7	18.2	-\$0.24 ± 0.16 <sup>a</sup>	\$4.12 ± 0.15 <sup>b</sup>	\$2.35 ± 0.14 <sup>c</sup>
Angus x Brahman <sup>¶</sup>	10.3	16.1	9.3	\$0.55 ± 0.13 <sup>a</sup>	\$1.47 ± 0.13 <sup>b</sup>	\$3.03 ± 0.19 <sup>c</sup>
Angus x Charolais <sup>§</sup>	1.9	2.4	3.4	-\$0.87 ± 0.30 <sup>a</sup>	\$2.97 ± 0.32 <sup>b</sup>	\$1.13 ± 0.32 <sup>c</sup>
Angus x Charolais x Hereford	0.2	0.5	0.5	-\$0.17 ± 0.87 <sup>a</sup>	\$4.79 ± 0.75 <sup>b</sup>	\$4.03 ± 0.84 <sup>b</sup>
Angus x Hereford <sup>§</sup>	5.1	7.2	8.1	\$0.69 ± 0.19 <sup>a</sup>	\$3.83 ± 0.19 <sup>b</sup>	\$2.45 ± 0.20 <sup>c</sup>
Angus x Hereford x ¼ Brahman	1.1	1.1	2.0	\$0.53 ± 0.40 <sup>a</sup>	\$1.36 ± 0.47 <sup>a,b</sup>	\$2.81 ± 0.42 <sup>b</sup>
Angus x Limousin	0.7	0.4	0.8	\$2.66 ± 0.49 <sup>a</sup>	\$2.71 ± 0.78 <sup>a</sup>	\$1.31 ± 0.67 <sup>a</sup>
Angus x Simmental	0.3	0.1	0.2	-\$2.33 ± 0.76 <sup>a</sup>	-\$1.81 ± 1.84 <sup>a</sup>	-\$0.20 ± 1.38 <sup>a</sup>
Brahman	1.1	1.0	0.4	-\$11.54 ± 0.40 <sup>a</sup>	-\$8.31 ± 0.52 <sup>b</sup>	-\$12.56 ± 0.88 <sup>a</sup>
½ Brahman Cross	0.9	1.5	0.5	-\$0.58 ± 0.44 <sup>a</sup>	-\$2.57 ± 0.42 <sup>b</sup>	-\$1.67 ± 0.82 <sup>a,b</sup>
¼ Brahman Cross	7.2	5.2	6.6	-\$3.64 ± 0.16 <sup>a</sup>	-\$3.14 ± 0.22 <sup>a</sup>	-\$2.05 ± 0.23 <sup>b</sup>
Brahman x Hereford	0.5	0.8	0.4	-\$6.30 ± 0.62 <sup>a</sup>	-\$3.48 ± 0.56 <sup>b</sup>	-\$4.46 ± 0.95 <sup>a,b</sup>
Brahman x Limousin	0.4	0.6	0.1	-\$2.23 ± 0.67 <sup>a</sup>	-\$5.50 ± 0.65 <sup>b</sup>	-\$5.73 ± 1.87 <sup>a,b</sup>
Brangus <sup>¶</sup>	6.4	4.7	9.7	-\$1.36 ± 0.17 <sup>a</sup>	\$0.55 ± 0.23 <sup>b</sup>	-\$1.30 ± 0.19 <sup>a</sup>
Charolais	17.3	12.8	16.9	\$0.97 ± 0.10 <sup>a</sup>	\$1.25 ± 0.14 <sup>a</sup>	\$0.22 ± 0.14 <sup>b</sup>
Charolais x Angus x ¼ Brahman	0.4	0.6	1.4	-\$0.71 ± 0.68 <sup>a</sup>	\$0.95 ± 0.65 <sup>a,b</sup>	-\$1.44 ± 0.50 <sup>a,c</sup>
Charolais x Brahman	0.7	2.6	0.6	-\$4.23 ± 0.49 <sup>a</sup>	-\$1.20 ± 0.31 <sup>b</sup>	-\$0.19 ± 0.75 <sup>b</sup>
Charolais x ¼ Brahman	2.2	2.9	2.4	-\$0.80 ± 0.29 <sup>a</sup>	\$0.67 ± 0.30 <sup>b</sup>	-\$2.85 ± 0.38 <sup>c</sup>
Charolais x Limousin	3.8	4.5	1.3	\$2.93 ± 0.22 <sup>a</sup>	\$2.66 ± 0.24 <sup>a</sup>	\$0.35 ± 0.51 <sup>b</sup>
Hereford	2.5	1.5	5.1	-\$9.79 ± 0.27 <sup>a</sup>	-\$9.13 ± 0.42 <sup>a</sup>	\$1.70 ± 0.26 <sup>b</sup>
Hereford x Brahman x Angus	3.4	3.4	0.3	\$0.46 ± 0.23 <sup>a</sup>	\$2.25 ± 0.27 <sup>b</sup>	\$1.56 ± 1.01 <sup>a,b</sup>
Hereford x ¼ Brahman	1.2	1.8	0.9	-\$3.01 ± 0.38 <sup>a</sup>	-\$1.77 ± 0.38 <sup>a</sup>	-\$2.89 ± 0.60 <sup>a</sup>
Hereford x Charolais	1.9	1.7	1.3	\$1.93 ± 0.31 <sup>a</sup>	\$2.48 ± 0.38 <sup>a</sup>	\$0.99 ± 0.52 <sup>a</sup>
Hereford x Limousin <sup>#</sup>	5.4	2.6	0.9	\$0.63 ± 0.18 <sup>a</sup>	\$0.24 ± 0.31 <sup>a,b</sup>	-\$1.08 ± 0.63 <sup>b</sup>
Hereford x Simmental	0.7	0.4	0.2	-\$2.00 ± 0.51 <sup>a</sup>	-\$2.60 ± 0.85 <sup>a</sup>	-\$3.53 ± 1.49 <sup>a</sup>
Limousin <sup>#</sup>	11.7	8.7	6.9	\$0.80 ± 0.12 <sup>a</sup>	-\$0.48 ± 0.17 <sup>b</sup>	\$0.15 ± 0.22 <sup>b</sup>
Limousin x ¼ Brahman	1.6	2.5	0.5	-\$1.36 ± 0.34 <sup>a</sup>	-\$1.90 ± 0.32 <sup>a</sup>	-\$5.62 ± 0.80 <sup>b</sup>
Longhorn	0.3	0.2	< 0.1	-\$18.39 ± 0.81 <sup>a</sup>	-\$25.96 ± 1.05 <sup>b</sup>	-\$35.99 ± 2.33 <sup>c</sup>
Saler	0.9	0.5	0.5	-\$4.63 ± 0.45 <sup>a</sup>	-\$6.84 ± 0.69 <sup>b</sup>	-\$10.61 ± 0.85 <sup>c</sup>
Simmental <sup>#</sup>	3.1	0.9	0.5	-\$4.96 ± 0.24 <sup>a</sup>	-\$5.12 ± 0.52 <sup>a</sup>	-\$8.01 ± 0.80 <sup>b</sup>

<sup>†</sup> Mean selling price for 2000, 2005 and 2010 was \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>‡</sup> LS means ± SE.

<sup>§</sup> The frequency percentage of Angus, Angus x Charolais and Angus x Hereford calves increased from 2000 to 2010 ( $P < 0.01$ ).

<sup>¶</sup> The frequency percentage of Angus x Brahman and Brangus calves changed from 2000 to 2010 ( $P < 0.01$ ).

<sup>#</sup> The frequency percentage of Hereford x Limousin, Limousin and Simmental calves decreased from 2000 to 2010 ( $P < 0.01$ ).

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).

**Table 3. Calf color frequency distribution and the 2000, 2005 and 2010 Arkansas selling price reported as deviations from the respective means.**

Calf color	Frequency percentage <sup>†</sup>			Deviation from the respective means <sup>‡,§</sup>		
	2000	2005	2010	2000	2005	2010
Black <sup>¶</sup>	26.6	38.6	45.0	-\$0.01 ± 0.08 <sup>a</sup>	\$1.86 ± 0.08 <sup>b</sup>	\$1.70 ± 0.09 <sup>b</sup>
Black-white face <sup>¶</sup>	9.8	11.1	11.9	\$0.68 ± 0.14 <sup>a</sup>	\$2.62 ± 0.16 <sup>b</sup>	\$3.01 ± 0.18 <sup>b</sup>
Gray <sup>¶</sup>	4.9	7.7	7.8	-\$2.36 ± 0.20 <sup>a</sup>	\$0.58 ± 0.19 <sup>b</sup>	-\$1.38 ± 0.22 <sup>c</sup>
Gray-white face	1.3	1.0	0.7	-\$3.34 ± 0.38 <sup>a</sup>	-\$1.05 ± 0.54 <sup>b</sup>	-\$3.83 ± 0.71 <sup>a</sup>
Red <sup>#</sup>	16.7	12.4	8.5	-\$1.16 ± 0.11 <sup>a</sup>	-\$2.95 ± 0.15 <sup>b</sup>	-\$3.68 ± 0.21 <sup>c</sup>
Red-white face <sup>#</sup>	12.2	7.2	5.0	-\$2.61 ± 0.12 <sup>a</sup>	-\$2.33 ± 0.19 <sup>a</sup>	-\$3.44 ± 0.27 <sup>b</sup>
Spotted or striped	2.6	1.9	1.4	-\$8.79 ± 0.27 <sup>a</sup>	-\$8.84 ± 0.38 <sup>a</sup>	-\$14.58 ± 0.51 <sup>b</sup>
White <sup>#</sup>	7.8	5.9	5.3	\$0.75 ± 0.16 <sup>a</sup>	-\$1.02 ± 0.21 <sup>b</sup>	-\$3.35 ± 0.26 <sup>c</sup>
Yellow	12.7	10.5	11.8	\$1.54 ± 0.12 <sup>a</sup>	\$2.59 ± 0.16 <sup>b</sup>	\$1.81 ± 0.18 <sup>a</sup>
Yellow-white face <sup>#</sup>	5.4	3.7	2.6	\$0.51 ± 0.19 <sup>a</sup>	\$2.56 ± 0.27 <sup>b</sup>	\$0.87 ± 0.37 <sup>a</sup>

<sup>†</sup> The frequency percentage changes from 2000 to 2005 were different ( $P < 0.01$ ) for all calf colors.

<sup>‡</sup> Mean selling price for 2000, 2005 and 2010 was \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>§</sup> LS means ± SE.

<sup>¶</sup> The frequency percentage of black, black-face and grey calves increased from 2000 to 2010 ( $P < 0.01$ ).

<sup>#</sup> The frequency percentage of red, red-white face, white and yellow-faced calves decreased 2000 to 2010 ( $P < 0.01$ ).

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).

**Table 4. Horned status frequency distribution and the 2000, 2005 and 2010 Arkansas selling price reported as deviations from the respective means.**

Horn status	Frequency percentage <sup>†</sup>			Deviation from the respective mean <sup>‡,§</sup>		
	2000	2005	2010	2000	2005	2010
Horns	25.1	13.1	9.2	-\$1.17 ± 0.09 <sup>a</sup>	-\$2.48 ± 0.14 <sup>b</sup>	-\$4.25 ± 0.20 <sup>c</sup>
Polled	74.9	86.9	90.8	-\$0.45 ± 0.05 <sup>a</sup>	\$1.15 ± 0.06 <sup>b</sup>	\$0.93 ± 0.06 <sup>c</sup>

<sup>†</sup> The frequency percentage of calves with horns decreases from 2000 to 2010 ( $P < 0.01$ ).

<sup>‡</sup> Mean selling prices for 2000, 2005 and 2010 was \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>§</sup> LS means ± SE.

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).

**Table 5. Frame score frequency distribution and the 2000, 2005 and 2010 Arkansas selling price reported as deviations from the respective means.**

Frame score	Frequency percentage			Deviation from the respective means <sup>‡,§</sup>		
	2000	2005	2010	2000	2005	2010
Large <sup>†</sup>	55.9	65.9	60.3	-\$0.84 ± 0.06 <sup>a</sup>	\$0.49 ± 0.06 <sup>b</sup>	\$0.74 ± 0.08 <sup>c</sup>
Medium	43.1	33.5	39.0	-\$0.05 ± 0.07 <sup>a</sup>	\$1.32 ± 0.09 <sup>b</sup>	\$0.24 ± 0.10 <sup>a</sup>
Small	1.0	0.6	0.8	-\$16.54 ± 0.44 <sup>a</sup>	-\$17.84 ± 0.65 <sup>a</sup>	-\$16.42 ± 0.67 <sup>a</sup>

<sup>†</sup> The frequency percentage of large-framed calves differs among years ( $P < 0.01$ ).

<sup>‡</sup> Mean selling prices for 2000, 2005 and 2010 was \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>§</sup> LS means ± SE.

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).



**Table 6. Muscle score frequency distribution and the 2000, 2005 and 2010 Arkansas selling price reported as deviations from the respective means.**

Muscle score	Frequency percentage <sup>†</sup>			Deviation from the respective means <sup>‡, §</sup>		
	2000	2005	2010	2000	2005	2010
1	85.4	75.3	82.0	\$0.51 ± 0.04 <sup>a</sup>	\$2.75 ± 0.06 <sup>b</sup>	\$2.21 ± 0.06 <sup>c</sup>
2	14.7	23.5	17.2	-\$8.49 ± 0.11 <sup>a</sup>	-\$5.40 ± 0.10 <sup>b</sup>	-\$5.91 ± 0.13 <sup>c</sup>
3	0.4	1.0	0.7	-\$15.93 ± 1.16 <sup>a</sup>	-\$19.37 ± 0.45 <sup>b</sup>	-\$21.78 ± 0.59 <sup>c</sup>

<sup>†</sup> The frequency percentage changes for muscle score 1, 2 and 3 from 2000 to 2005 were different ( $P < 0.01$ ).

<sup>‡</sup> Mean selling prices for 2000, 2005 and 2010 was \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>§</sup> LS means ± SE.

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).

**Table 7. The 2000, 2005 and 2010 Arkansas selling price by weight group as reported deviations from the respective means.**

Weight group (lbs)	Deviation from the respective means <sup>†, ‡</sup>		
	2000	2005	2010
< 300	\$17.90 ± 0.32 <sup>a</sup>	\$9.80 ± 1.20 <sup>b</sup>	\$2.32 ± 1.45 <sup>c</sup>
300-349	\$15.29 ± 0.14 <sup>a</sup>	\$14.43 ± 0.20 <sup>b</sup>	\$10.13 ± 0.21 <sup>c</sup>
350-399	\$8.47 ± 0.11 <sup>a</sup>	\$10.98 ± 0.15 <sup>b</sup>	\$8.08 ± 0.17 <sup>a</sup>
400-449	\$3.69 ± 0.10 <sup>a</sup>	\$6.74 ± 0.12 <sup>b</sup>	\$4.44 ± 0.14 <sup>c</sup>
450-499	-\$1.08 ± 0.10 <sup>a</sup>	\$1.05 ± 0.12 <sup>b</sup>	\$0.74 ± 0.14 <sup>b</sup>
500-549	-\$4.79 ± 0.10 <sup>a</sup>	-\$2.59 ± 0.12 <sup>b</sup>	-\$1.90 ± 0.14 <sup>c</sup>
550-599	-\$7.98 ± 0.13 <sup>a</sup>	-\$6.53 ± 0.14 <sup>b</sup>	-\$5.63 ± 0.17 <sup>c</sup>
600-649	-\$10.40 ± 0.16 <sup>a</sup>	-\$9.83 ± 0.17 <sup>a</sup>	-\$7.01 ± 0.20 <sup>b</sup>
650-699	-\$12.87 ± 0.19 <sup>a</sup>	-\$13.27 ± 0.23 <sup>a</sup>	-\$10.15 ± 0.28 <sup>b</sup>
700-749	-\$15.37 ± 0.31 <sup>a</sup>	-\$16.04 ± 0.32 <sup>a</sup>	-\$12.59 ± 0.38 <sup>b</sup>
750-799	-\$17.02 ± 0.43 <sup>a</sup>	-\$20.64 ± 0.46 <sup>b</sup>	-\$15.42 ± 0.55 <sup>a</sup>
>800	-\$19.49 ± 0.52 <sup>a</sup>	-\$25.38 ± 0.59 <sup>b</sup>	-\$18.07 ± 0.62 <sup>a</sup>

<sup>†</sup> Mean selling price for 2000, 2005 and 2010 were \$93.94, \$117.00 and \$109.12/cwt., respectively.

<sup>‡</sup> LS means ± SE.

<sup>a,b,c</sup> Least squares means within each row without a common superscript differ ( $P < 0.01$ ).

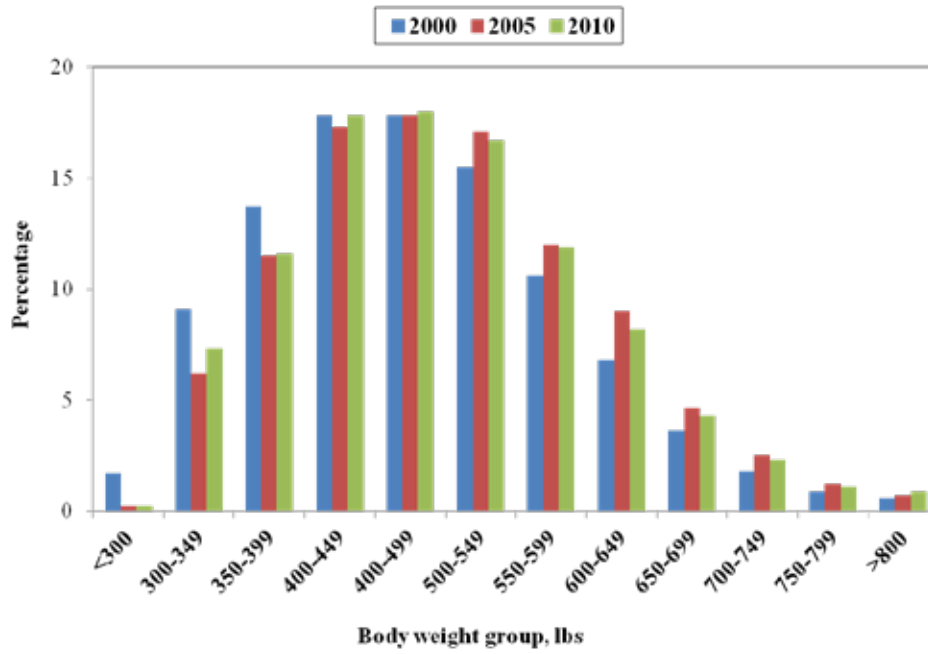


Fig. 1. The percentage of calves sold by weight groups in Arkansas livestock auctions in 2000, 2005 and 2010.

# Relationships of polymorphisms of lactate dehydrogenase to heifer immune response

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## Story in Brief

Objectives of this research were to determine the effects of concentration of lactate dehydrogenase enzyme on immune response and to determine the effects of single nucleotide polymorphisms of lactate dehydrogenase on immune response of replacement heifers grazing mixed stands of endophyte infected tall fescue. Angus based heifers (n = 89) underwent a 48 hour delayed hypersensitivity trial at 10 months of age. Heifers were injected with phytohemagglutinin in the caudal fold at 0 hour. Skin fold thickness was measured at 0, 12, 24 and 48 hours. Blood samples were taken at 24 and 48 hours post injection. Genomic deoxyribonucleic acid, prepared from buffy coat was sequenced for single nucleotide polymorphisms of lactate dehydrogenase using Sequenom technology. Single nucleotide polymorphisms of lactate dehydrogenase affected or tended to affect multiple blood cell markers at different times.

## Introduction

Financial loss from disease in beef cattle as a whole has not been established. Estimates of individual disease loss includes 7-10% decreased calf weaning weight in cows infected with mastitis (Brett, 1998) and \$720 loss for a cow infected with Johne's disease (Lents, 1997). Circulating amounts of lactate dehydrogenase (LDH) has been shown to increase with the presence of disease. Specific elevated isoenzyme activity has raised the sensitivity of testing to determine which portion of the body is involved. Isoenzyme patterns have also been used to determine the diagnosis of viral versus bacterial infection (Fishbach and Dunning III, 2009).

Therefore, the objective of this study was to determine effects of single nucleotide polymorphisms (SNP) of LDH on immune function in replacement heifers grazing mixed stands of endophyte infected fescue.

## Materials and Methods

The ten month old Angus-based heifers (n = 89) used for this trial were located at the University of Arkansas Beef Farm in Savoy, Ark. Heifers were maintained on endophyte infected tall fescue [*Lolium arundinaceum* (Shreb.)] and native grasses with free choice access to Pasture Mineral + Mag (Tri State Agri. Services, L.L.C., Afton, Okla.). Dry corn gluten feed was provided daily from 0800 to 0900 and hay was provided when pasture growth was not adequate to support nutritional requirements.

Heifers were vaccinated with a booster shot at weaning for Infectious Bovine Rhinotracheitis, Parainfluenza 3 and Bovine Viral Diarrhea (Pyramid 10<sup>®</sup>, Boehringer Ingelheim, St. Joseph, Mo.). They also received a 7 way clostridial booster (Alpha 7<sup>®</sup>, Boehringer Ingelheim, St. Joseph, Mo.). Heifers were dewormed with an oral drench (Synanthic<sup>®</sup>, Boehringer Ingelheim, St. Joseph, Mo.) two weeks post weaning. Heifer hair coat scores (CS) were assessed using a 1 to 5 scale at weaning (day 0), 12 months of age (day 108), and 15 months (day 177) as described by Turner and Schleger (1960).

Immunological effects of LDH single nucleotide polymorphisms were determined through cell mediated immunity via delayed type hypersensitivity testing. One mg phytohemagglutinin (PHA-M) (Sigma Aldrich, St. Louis, Mo.) diluted in 1 mL saline was administered intradermally in the caudal fold at 0 h. Skin fold thickness (SF) was measured at 0 h, 12 h, 24 h, and 48 h using micrometric

calipers (Vernier Software & Technology LLC., Beaverton, Ore.) (Ata, 2011). The mucoprotein form of PHA was employed for T cell activation of the CD3-Ti complex that is found on the surface of all T cells that causes an escalation in intracellular calcium for response (O'Flynn et al., 1986). In contrast, other forms of PHA elicit responses by targeting only the CD2+ receptor that is not found on all forms of T cells (Yang et al., 2001). The mucoprotein form of phytohemagglutinin thus provided greater accuracy of achieving complete immune response.

Blood samples were taken at 24 h and 48 h after PHA injection using purple top vacuum tubes (Vacutainer, Becton Dickinson, Inc., Franklin Lakes, N.J.) containing the anticoagulant EDTA. Blood cell concentrations were determined using a Hemavet 950 (Drew Scientific Inc., Oxford, Conn.) beginning a maximum of four hours post collection. Genomic DNA was extracted, purified, and sequenced. Blood components determined were white blood cells (WBC), red blood cells (RBC), neutrophils (NE), eosinophils (EO), lysosomes (LY), platelet count (PLT) and mean corpuscular volume (MCV).

The PROC GLM of SAS (SAS Inst. Inc., Cary, N.C.) was used to determine effects of time, SNP, and SNP by time interaction on blood cell concentrations and SF thickness. Means were separated using repeated *t*-test in the PDIFF Option of the GLM Procedure of SAS.

## Results and Discussion

Five SNP appeared in the LDHB promoter and coding sequences (Table 1). Results indicate SNP G163A affected the most immune response measurements (Table 2). Heterozygous heifers (GA) had increased WBC, NE, and RBC ( $P < 0.05$ ) compared to homozygous heifers. Heterozygous heifers (GA) had increased WBC, NE, and RBC ( $P < 0.05$ ) compared to homozygous heifers. Heterozygous animals also had increased EO counts at 24 h and 48 h ( $P < 0.01$ ).

Homozygous heifers (CC) at bp 669 (Table 3) had elevated MCV ( $P < 0.05$ ) when compared to heterozygous counterparts. Platelet count tended to be higher for homozygous animals at 24 h and 48 h ( $P < 0.15$ ). Heifers heterozygous (CT) at bp 669 displayed a tendency for increased WBC at 24 h and LY, a subdivision of WBC, at 24 and 48 h ( $P < 0.15$ ).

Data in Table 4 indicates LDHB SNP A606G was related to 48 h MCV and 24 h PLT ( $P < 0.05$ ). Heifers homozygous for

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the major allele (AA) had higher counts. White blood cell count, lymphocytes, and platelet count (48 h) tended to be affected ( $P < 0.15$ ). Heterozygous animals (CT) had elevated WBC and LY, whereas homozygous animals had raised PLT count.

The remaining two SNPs: Single nucleotide polymorphisms C541A and G348A were moderately related to immune response (Table 5 and 6). C541A was correlated to 48 h PLT ( $P < 0.05$ ) where homozygous heifers (CC) had raised cell counts. SNP at base position 348 tended to affect both MCV (24 and 48 h) and 48 h PLT ( $P < 0.10$ ). Heifers homozygous for the major allele (GG) had higher PLT, but lower MCV. Regardless of SNP, MCV levels were below normal range. Skin fold thickness at 24 h had tended to be affected ( $P < 0.15$ ) by the bp 348 SNP. Heterozygous (GA) animals had the thickest SF.

Genotypes of LDHB were related to WBC count at specific base pair. Type of WBC cellular increases were associated with different CS (data not shown). Animals in which CS was higher subsequently had greater NE counts, which could be attributed to adrenaline release.

In cases where WBC increased, but CS was similar among genotypes, lymphocytes increased. Elevation in LY numbers may have been associated with raised respiratory rates to dissipate heat as temperatures rose during working conditions.

### Implications

Genetic selection for immune response would assist in choosing desirable replacement heifers. Using lactate dehydrogenase B single

nucleotide polymorphisms, producers may well target heifers with greater immediate and sustained immunity.

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**Table 1. Allelic percentages and genotypic frequencies of LDHB promoter and coding sequence single nucleotide polymorphisms (SNP).**

Mutation	Allele %		Genotype, <i>n</i>	
	G	A	GG	G
G163A <sup>†</sup>	96.8	3.2	74	5
G348A <sup>†</sup>	57.0	43.0	G 24	GA AA
C541A <sup>‡</sup>	94.3	5.7	78	10
A606G <sup>‡</sup>	94.9	5.1	79	9
C669T <sup>‡</sup>	95.0	5.0	80	9

<sup>†</sup> Promoter region SNP.

<sup>‡</sup> Coding sequence SNP.

**Table 2. Lactate dehydrogenase B G163A SNP relationship to immune response measures.**

Measurement	Time (h)	Genotypic Averages	
		GG	GA
Skin Fold Thickness (mm)	24	5.82 ± 1.07	5.87 ± 0.62
	48	3.95 ± 0.76	3.75 ± 1.32
White Blood Cell (K/μL)	24	11.70 <sup>c</sup> ± 2.46	15.09 <sup>d</sup> ± 3.60
	48	11.13 <sup>c</sup> ± 2.28	13.76 <sup>d</sup> ± 2.59
Neutrophil (K/μL)	24	4.01 <sup>c</sup> ± 0.93	7.37 <sup>d</sup> ± 3.18
	48	3.88 <sup>c</sup> ± 1.03	6.06 <sup>d</sup> ± 1.89
Lymphocytes (K/μL)	24	6.62 ± 1.66	5.81 ± 1.17
	48	6.34 ± 1.50	6.20 ± 1.53
Red Blood Cell (M/μL)	24	8.98 <sup>c</sup> ± 0.79	9.88 <sup>d</sup> ± 0.68
	48	8.95 <sup>c</sup> ± 0.85	10.05 <sup>d</sup> ± 0.85
Mean Corpuscular Volume (fL)	24	32.77 ± 2.75	31.07 ± 2.02
	48	32.68 ± 2.87	31.17 ± 2.10
Platelet (K/μL)	24	451.78 ± 131.32	472.50 ± 128.28
	48	434.50 ± 115.94	484.50 ± 104.72
Eosinophil (K/μL)	24	0.53 <sup>a</sup> ± 0.27	1.19 <sup>b</sup> ± 0.35
	48	0.42 <sup>a</sup> ± 0.23	0.88 <sup>b</sup> ± 0.46

<sup>a,b</sup>*P* < 0.01.<sup>c,d</sup>*P* < 0.05.**Table 3. Lactate Dehydrogenase B C669T SNP relationship to immune response measures.**

Measurement	Time (h)	Genotypic Averages	
		CC	CT
Skin Fold Thickness (mm)	24	5.89 ± 1.08	5.56 ± 0.82
	48	3.95 ± 0.79	4.06 ± 0.77
White Blood Cell (K/μL)	24	11.73 <sup>c</sup> ± 2.40	13.11 <sup>d</sup> ± 3.34
	48	11.19 ± 2.24	12.03 ± 2.97
Neutrophil (K/μL)	24	4.17 ± 1.31	4.52 ± 1.15
	48	4.03 ± 1.24	4.06 ± 0.81
Lymphocytes (K/μL)	24	6.45 <sup>c</sup> ± 1.49	7.37 <sup>d</sup> ± 2.10
	48	6.21 <sup>c</sup> ± 1.35	6.98 <sup>d</sup> ± 2.02
Red Blood Cell (M/μL)	24	9.05 ± 0.84	9.22 ± 0.41
	48	9.06 ± 0.91	9.00 ± 0.55
Mean Corpuscular Volume	24	32.87 ± 2.67	31.02 ± 2.28
	48	32.82 <sup>a</sup> ± 2.74	30.75 <sup>b</sup> ± 2.43
Platelet (K/μL)	24	461.90 <sup>c</sup> ± 109.73	383.75 <sup>d</sup> ± 230.05
	48	446.48 <sup>c</sup> ± 98.23	371.87 <sup>d</sup> ± 186.32
Eosinophil (K/μL)	24	0.58 ± 0.30	0.58 ± 0.29
	48	0.47 ± 0.27	0.37 ± 0.19

<sup>a,b</sup>*P* < 0.05.<sup>c,d</sup>*P* < 0.15.

**Table 4. Lactate Dehydrogenase B A606G SNP relationships to immune response measures.**

Measurement	Time (h)	Genotypic Averages	
		AA	AG
Skin Fold Thickness (mm)	24	5.88 ± 1.08	5.56 ± 0.82
	48	3.93 ± 0.78	4.06 ± 0.77
White Blood Cell (K/μL)	24	11.72 ± 2.42 <sup>c</sup>	13.11 ± 3.34 <sup>d</sup>
	48	11.13 ± 2.20	12.03 ± 2.97
Neutrophil (K/μL)	24	4.17 ± 1.32	4.52 ± 1.15
	48	3.98 ± 1.17	4.06 ± 0.81
Lymphocytes (K/μL)	24	6.45 <sup>c</sup> ± 1.50	7.38 <sup>d</sup> ± 2.10
	48	6.22 ± 1.36	6.98 ± 2.02
Red Blood Cell (M/μL)	24	9.04 ± 0.85	9.22 ± 0.41
	48	9.05 ± 0.92	9.00 ± 0.55
Mean Corpuscular Volume (fL)	24	32.91 ± 2.67	31.02 ± 2.28
	48	32.86 <sup>a</sup> ± 2.72	30.75 <sup>b</sup> ± 2.43
Platelet (K/μL)	24	459.84 <sup>a</sup> ± 109.11	383.75 <sup>b</sup> ± 230.05
	48	445.60 <sup>c</sup> ± 98.64	371.87 <sup>d</sup> ± 186.32
Eosinophil (K/μL)	24	0.57 ± 0.30	0.58 ± 0.29
	48	0.46 ± 0.27	0.37 ± 0.19

<sup>a,b</sup>*P* < 0.05.<sup>c,d</sup>*P* < 0.15.**Table 5. Lactate Dehydrogenase B C541A SNP relationship to immune response measures.**

Measurement	Time (h)	Genotypic Averages	
		CC	CA
Skin Fold Thickness (mm)	24	5.90 ± 1.08	5.44 ± 0.84
	48	3.95 ± 0.78	3.94 ± 0.80
White Blood Cell (K/μL)	24	11.73 ± 2.43	12.90 ± 3.19
	48	11.13 ± 2.21	11.93 ± 2.79
Neutrophil (K/μL)	24	4.17 ± 1.33	4.49 ± 1.08
	48	3.97 ± 1.17	4.16 ± 0.81
Lymphocytes (K/μL)	24	6.46 ± 1.50	7.15 ± 2.08
	48	6.24 ± 1.36	6.75 ± 2.00
Red Blood Cell (M/μL)	24	9.06 ± 0.84	9.04 ± 0.65
	48	9.07 ± 0.91	8.85 ± 0.67
Mean Corpuscular Volume (fL)	24	32.82 ± 2.58	31.91 ± 3.40
	48	32.77 ± 2.65	31.68 ± 3.61
Platelet (K/μL)	24	458.94 ± 109.63	399.22 ± 220.14
	48	444.62 <sup>a</sup> ± 99.01	387.66 <sup>b</sup> ± 180.61
Eosinophil (K/μL)	24	0.57 ± 0.30	0.63 ± 0.32
	48	0.46 ± 0.26	0.41 ± 0.20

<sup>a,b</sup>*P* < 0.05.

**Table 6. Lactate Dehydrogenase B G348A SNP relationship to immune response measures.**

Measurement	Time (h)	Genotypic Averages		
		GG	GA	AA
Skin Fold Thickness (mm)	24	5.88 <sup>c</sup> ± 1.03	5.97 <sup>c</sup> ± 1.06	5.22 <sup>d</sup> ± 0.90
	48	4.07 ± 1.00	3.91 ± 0.72	3.95 ± 0.65
White Blood Cell (K/μL)	24	12.41 ± 2.53	11.72 ± 2.41	11.48 ± 3.13
	48	12.01 ± 2.12	11.07 ± 2.34	10.80 ± 2.55
Neutrophil (K/μL)	24	4.39 ± 1.93	4.15 ± 0.95	4.02 ± 1.18
	48	4.33 ± 1.48	3.96 ± 1.13	3.75 ± 0.91
Lymphocytes (K/μL)	24	6.89 ± 1.03	6.44 ± 1.70	6.34 ± 1.90
	48	6.69 ± 0.75	6.15 ± 1.61	6.19 ± 1.62
Red Blood Cell (M/μL)	24	9.13 ± 0.91	8.99 ± 0.83	9.26 ± 0.51
	48	9.17 ± 0.91	8.94 ± 0.95	9.23 ± 0.80
Mean Corpuscular Volume (fL)	24	31.73 <sup>a</sup> ± 1.92	33.19 <sup>b</sup> ± 3.01	32.57 <sup>a,b</sup> ± 1.96
	48	31.70 <sup>a</sup> ± 1.85	33.09 <sup>b</sup> ± 3.12	32.48 <sup>a,b</sup> ± 2.38
Platelet (K/μL)	24	469.09 ± 91.74	464.48 ± 140.26	384.81 ± 114.75
	48	464.00 <sup>a</sup> ± 61.31	444.97 <sup>a</sup> ± 122.74	372.00 <sup>b</sup> ± 114.60
Eosinophil (K/μL)	24	0.57 ± 0.34	0.59 ± 0.27	0.56 ± 0.33
	48	0.49 ± 0.33	0.47 ± 0.25	0.35 ± 0.18

<sup>a,b</sup>*P* < 0.10.<sup>c,d</sup>*P* < 0.15.

# Evaluation of hair shedding scores in relation to maternal traits and productivity in beef cattle

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## Story in Brief

The objective of this study was to measure variation in hair coat shedding to determine relationships to production traits. During 28-day intervals, crossbred-Angus cows ( $n = 199$ ) were observed over a five-month period and evaluated for coat shedding on a 5 point scale. A score of 5 indicated a full winter coat and 1 represented a slick, short summer coat. For each cow, the first month a score of 3 or less was reached (at least 50% shed) was considered the month of first shedding (MFS). Data including calf weaning weight, body condition score (BCS) of cow at weaning, body weight (BW) of cow at weaning, BCS of cow pre-breeding, BW of cow pre-breeding, pregnancy rate, birth weight of calf and age of the cow were collected and analyzed in PROC MIXED and FREQ of SAS. Frequency for MFS was highest for June, followed by May, July and April, respectively. Calf birth weight was highest ( $P = 0.015$ ) for cows exhibiting MFS in May and lowest for cows exhibiting MFS in July. Calf weaning weight was similar ( $P = 0.8$ ) among MFS categories with April, May, June and July cows exhibiting calf weaning weights of 493, 471, 471 and 455 lb, respectively. Cow body weight at weaning was highest ( $P = 0.05$ ) in cows exhibiting MFS in May (1193 lb) and lowest in cows with MFS in June (1046 lb). No differences were noted in BCS of cows at weaning or in BCS of cows pre-breeding. Cow body weight at pre-breeding was highest ( $P = 0.01$ ) for cows exhibiting MFS in May (1185 lb) and lowest in cows with MFS in June (960 lb). In these data, shedding of winter hair coats were related to maternal body weight at two different points during production as well as related to calf birth weight.

## Introduction

Long, thick and dark hair coats improve conservation and maintenance of body heat (Gray et al., 2011). However, during periods of high temperatures and humidity, cattle are susceptible to heat stress. If cattle overheat, issues with decreased fertility, milk production, and growth can occur (Bilby et al., 2008).

Cattle dissipate heat mainly through evaporative cooling during respiration and through sweating. Heat dissipation is essential to maintaining normal production and to lessen the chance of heat stress. Elevated environmental temperatures could negatively affect cattle with thick, wooly coats drastically more than those with slick, short summer coats. In the sub-tropical climate of the southeastern United States, cattle that do not shed their winter coat efficiently exhibit signs of impaired production traits such as reduced calf weaning weights likely due to heat stress (Gray et al., 2011).

The objectives of this study were to measure the rate at which cattle shed their winter coat in the warm, humid climate of Arkansas, and to determine if any relationship existed between production parameters and coat shedding activity.

## Materials and Methods

Angus crossbred cows and heifers ( $n = 199$ ) were used for this study. Cattle were located at the University of Arkansas' beef cattle research unit near Savoy, Ark. Observations were made on the study animals from March through July of 2012. Cows ranged from 2 to 16 years of age. All mature cows weaned a calf in May 2012, and if pregnant, were scheduled to calve during the fall of 2012 and rebreeding began in November 2012. Phenotypic traits collected included cow body weight at weaning, body condition score (BCS; Richards et al., 1986) at weaning, cow body weight at prebreeding, BCS at prebreeding, pregnancy rate, calf birth weight and calf weaning weight.

Hair shedding scores were collected monthly by a trained panel of university faculty and staff based on a 1 to 5 coat shedding scale (Table 1) adapted from Gray et al. (2011). The first month a score of 3 (50% shed) or less was reached was considered the month of first shedding (MFS). Four levels of MFS were noted: April, May, June and July. For each cow, association between MFS and phenotypic data (weights, BCS, pregnancy status, etc.) were analyzed utilizing the FREQ and MIXED procedures of SAS (SAS Inst. Inc., Cary, N.C.). Statistical significance was considered for a  $P$ -value of less than or equal to 0.05.

## Results and Discussion

As temperatures increased during spring and summer months, cattle began shedding of winter hair coats. In March, all cattle maintained a winter coats and shedding score of 5, however, by July study animals had all displayed a shedding score of  $\leq 3$ . Figure 1 displays percent of cows exhibiting a hair shedding score of  $\leq 3 \times$  month.

Age and MFS exhibited a significant relationship ( $P \leq 0.05$ ; Fig. 2). Average age of cows reaching MFS was 3.75, 7.5, 5.2, and 2.7 years for April, May, June, and July, respectively. Cow body weight at weaning was highest ( $P = 0.05$ ; Fig. 3) in cows exhibiting MFS in May (1193 lb) and lowest in cows with MFS in June (1046 lb). No differences were noted in BCS of cows at weaning ( $P = 0.44$ ; Fig. 4) or in BCS of cows pre-breeding ( $P = 0.97$ ; Fig. 6). Cow BW at pre-breeding was highest ( $P = 0.01$ ; Fig. 5) for cows exhibiting MFS in May (1185 lb) and lowest in cows with MFS in June (960 lb). Shedding of the winter hair coats were noted to be related to maternal BW at two different points during the production and year, and cow body weight was closely associated with the age of the cows with 2 and 3 year old cows exhibiting lighter BW compared to older cows. Pregnancy rates were similar ( $P = 0.21$ ) for all MFS categories with April, May, June and July groups exhibiting pregnancy rates of 75, 100, 81 and 100 percent, respectively.

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Calf birth weight was highest ( $P = 0.015$ ; Fig. 7) for cows exhibiting MFS in May and lowest for cows exhibiting MFS in July at 82.6 and 66.8 pounds, respectively. Calf weaning weight was similar ( $P = 0.8$ ; Fig. 8) for all MFS categories.

### Implications

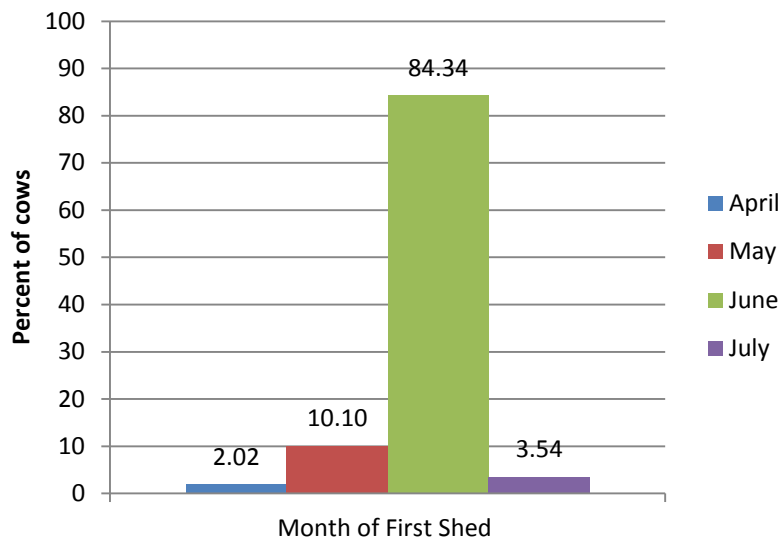
Winter hair coat shedding for the study herd occurred over a four-month period between April and July with the bulk of the animals shedding in June. Month that shedding scores reached 3 or lower was associated with cow body weight at weaning, cow body weight pre-breeding and calf birth weight. Additional research is needed to confirm the relationship between hair coat shedding score and these phenotypic data, and possible mechanisms governing that association.

### Literature Cited

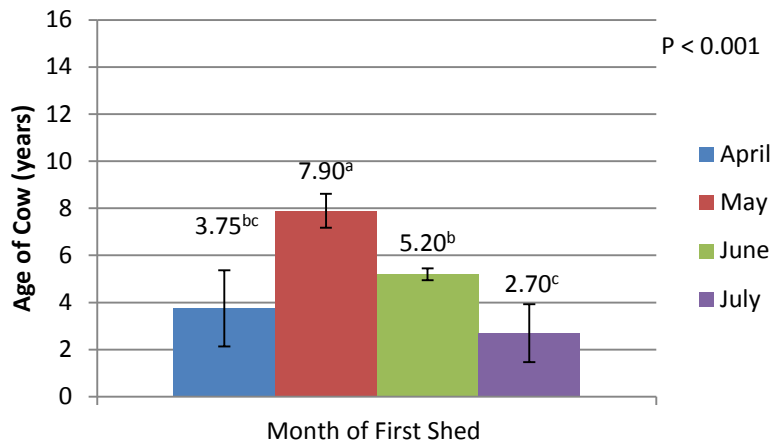
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**Table 1. Hair coat shedding score scale.**

Hair coat shedding score	Explanation
5	Thick winter coat (0% shed)
4	Shedding has begun (25% shed)
3	Half of shedding is complete (50% shed)
2	Most of shedding is complete (75% shed)
1	Slick summer coat (100% shed)

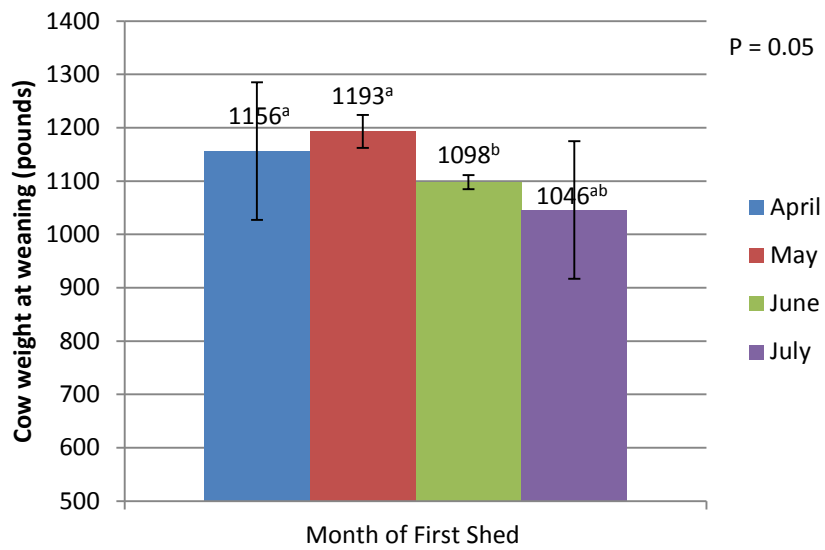


**Fig. 1. Percentage of cattle reaching month of first shedding score by month.**



<sup>a-c</sup> Least-squares means with differing superscripts differ.

Fig. 2. Cow age by month of first shedding.



<sup>a-b</sup> Least-squares means with differing superscripts differ.

Fig. 3. Cow weight at weaning by month of first shedding.

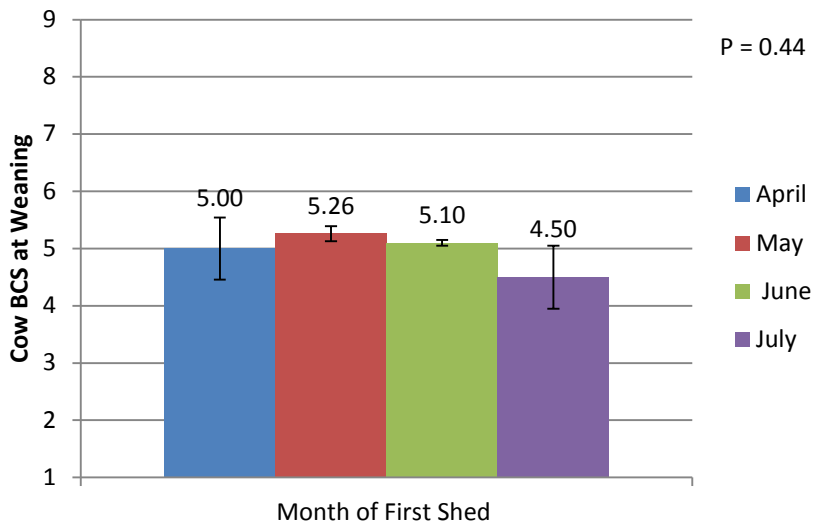
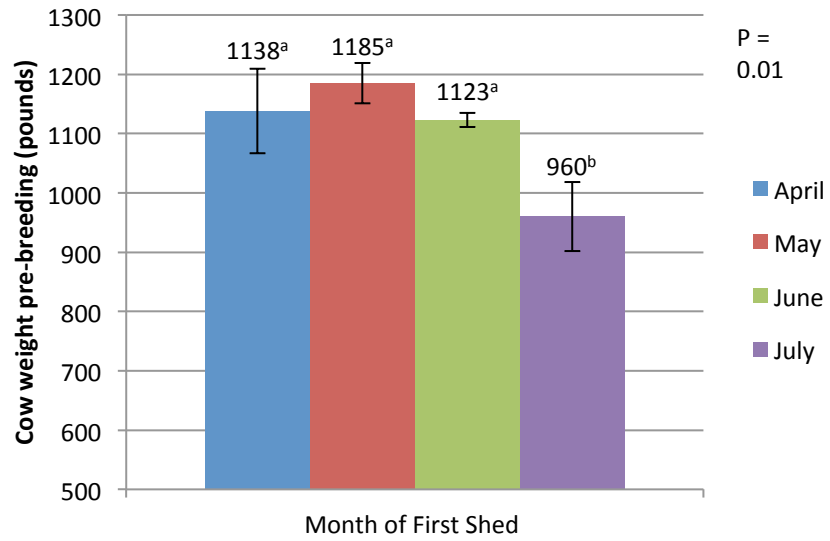


Fig. 4. Cow BCS at weaning by month of first shedding.



<sup>a-b</sup> Least-squares means with differing superscripts differ.

Fig. 5. Cow body weight pre-breeding by month of first shedding.

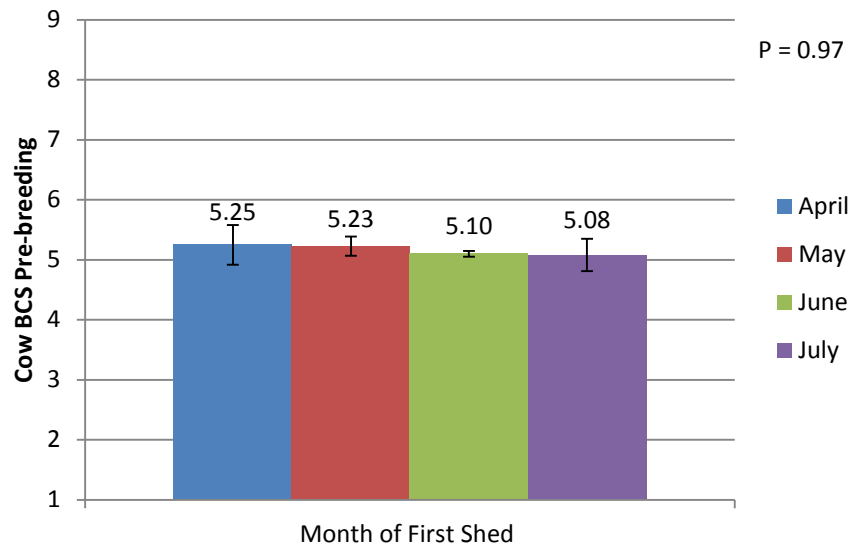
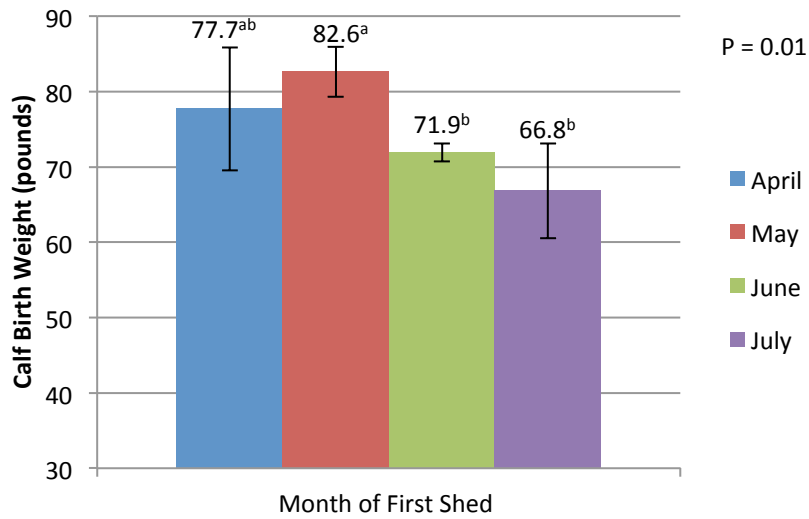


Fig. 6. Cow BCS pre-breeding by month of first shedding.



<sup>a-b</sup> Least-squares means with differing superscripts differ.

Fig. 7. Calf birth weight by month of first shedding.

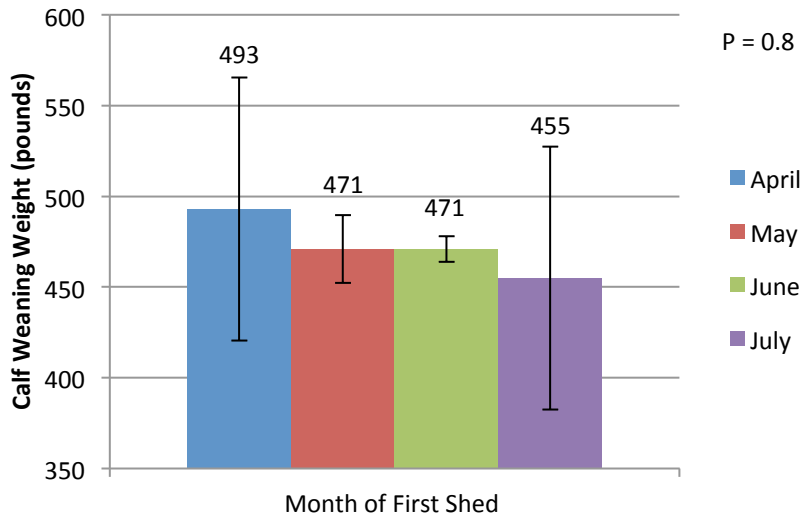


Fig. 8. Calf weaning weight by month of first shedding.

# Performance by yearling Katahdin ewes grazing tall fescue pastures using continuous or rotational grazing schemes—2 year summary

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## Story in Brief

Rotational grazing has increased in popularity in recent years; however, this grazing method has not been well documented in Katahdin hair sheep. Our objective was to evaluate the effects of continuous or rotational grazing methods on body weight, reproduction, and parasite infestation measurements by yearling Katahdin ewes grazing endophyte-infected tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] through late spring and summer. Over 2 consecutive years, a total of 50 yearling Katahdin ewes ( $116 \pm 1.5$  lb initial body weight ;  $3.6 \pm 0.11$  initial body condition score) were stratified by body weight and allocated randomly to 1 of 5, 1-acre toxic tall fescue pastures consisting of 2 treatments: 1) continuous (5 replications) or 2) 4-cell rotation (5 replications), yearly in early May. In mid-May, 1 ram was placed in each pasture for a 40-day breeding season. At breeding, end of breeding, and final body weight, average daily gain, and total gain did not differ ( $P \geq 0.19$ ) between treatments. At breeding body condition scores tended ( $P = 0.10$ ) to be greater for continuous compared with four-cell rotation. End of breeding and final body condition scores and at breeding, end breeding, and final FAMACHA<sup>®</sup> scores did not differ ( $P \geq 0.12$ ) across treatments. Lambing rates and frequency of multiple births were greater ( $P \leq 0.04$ ) from 4-cell rotation compared with continuous, but number of lambs per ewe exposed did not differ ( $P = 0.14$ ) across treatments. Therefore, utilizing rotational grazing schemes in late spring through summer by yearling Katahdin ewes may not increase performance, lower parasite infestation, or improve number of lambs per ewe exposed, but may increase lambing rates and frequency of multiple births.

## Introduction

Rotational grazing has been reported to have added benefits when compared with continuous grazing methods. These benefits include an increase in carrying capacity, better forage persistence and utilization, and better manure distribution (Ball et al., 2007), all of which has contributed to rotational grazing becoming popular among livestock producers in recent years. However, little research has been documented evaluating the effects of rotational compared with continuous grazing in sheep on endophyte-infected tall fescue (E+), especially using Katahdin hair sheep. Therefore, the objective of this study was to evaluate the effects of continuous or rotational grazing methods on body weight, reproduction, and parasite infestation measurements by yearling Katahdin ewes grazing E+ through late spring and summer.

## Materials and Methods

This study was conducted at Lincoln University Carver Farm in Jefferson City, Mo. Over 2 consecutive years, yearling Katahdin ewes [ $116 \pm 1.5$  lb initial body weight (BW);  $3.6 \pm 0.11$  initial body condition score (BCS)] were stratified by BW and allocated randomly (May 5, 2011 and May 7, 2012) to 1 of 5, 1-acre pastures consisting predominately of E+. Pastures were assigned randomly to 1 of 2 treatments consisting of: 1) continuous grazing (C; 5 replications) or 2) 4-cell rotation grazing (4R; 5 replications). At initiation of the study (14 days prior to breeding season) a Control Internal Drug Releasing (CIDR<sup>®</sup>; Pfizer Inc., New York, N.Y., Product No. 036116) device was placed intra-vaginally in each ewe. Beginning May 19, 2011 and May 21, 2012, every CIDR<sup>®</sup> was removed and each

ewe was administered 400 IU of PG600 (Intervet Inc., Millsboro, Del. 19966, Product No. 057174). At this time 1 ram that had passed a breeding soundness exam was placed in each pasture for a 40-day breeding season. Start breeding, end breeding, and final BW, BCS (1-5 scale; 1 = healthy; 5 = obese; Russel et al., 1969), and FAMACHA<sup>®</sup> scores (1-5 scale; 1 = healthy; 5 = severely anemic; indicator of parasite burden; Bath et al., 2001) were determined. Reproductive measurements included lambing rates, number of lambs born per ewe exposed, and frequency of multiple births.

Performance measurements were analyzed using PROC MIXED of SAS, with pasture as the experimental unit. Lambing rates and frequency of multiple births were analyzed by Chi-square using PROC FREQ of SAS (SAS Institute, Inc., Cary, N.C.). All data are reported as least squares means.

## Results and Discussion

Body weight and FAMACHA<sup>®</sup> scores at breeding, end of breeding, and at the end of the study did not differ ( $P \geq 0.12$ ) across treatments (Table 1). Ewe total gain and average daily gain (ADG) did not differ ( $P \geq 0.19$ ) for 4R compared with C, which agrees with previous work by Sharrow and Krueger (1979). Ewe BCS at breeding tended ( $P = 0.10$ ) to be greater for C compared with 4R, but did not differ ( $P \geq 0.44$ ) at the end of breeding and at the end of the study. Lambing rates and frequency of multiple births were greater ( $P \leq 0.04$ ) from 4R compared with C, but number of lambs born per ewe exposed did not differ ( $P = 0.14$ ) across treatments. Therefore, rotational grazing yearling Katahdin hair sheep in late spring through summer may not improve BW, BCS, or FAMACHA<sup>®</sup> scores but may increase lambing rates and frequency of multiple births.

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## Implications

Based on these results, producers may increase lambing rates and frequency of multiple births from yearly Katahdin hair sheep by switching from continuous to rotational grazing on endophyte-infected tall fescue, thus allowing more lambs to be sold.

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**Table 1. Performance by yearling Katahdin ewes grazing endophyte-infected tall fescue pastures using either a continuous or 4-cell rotation grazing scheme.**

Item	Treatment <sup>a</sup>		SEM <sup>b</sup>	P-Value
	C	4R		
Body weight, lb				
at breeding	120	118	5.7	0.66
end of breeding	126	125	7.3	0.83
end of study	131	132	10.3	0.66
BCS <sup>c</sup>				
at breeding	3.5	3.1	0.37	0.10
end of breeding	3.3	3.2	0.24	0.44
end of study	3.1	3.1	0.10	0.45
FAMACHA <sup>d</sup>				
at breeding	1	1	0.1	0.17
end of breeding	1	1	0.1	0.12
end of study	1	1	0.1	0.79
Average daily gain, lb	0.15	0.16	0.032	0.48
Total gain, lb	12.5	14.8	4.51	0.19
Lambing rate, % <sup>e</sup>	48	80	–	0.03
Number of lambs/ewe exposed	0.8	1.3	0.34	0.14
Frequency of multiple births, % <sup>e</sup>	28	56	–	0.04

<sup>a</sup>C = Continuous; 4R = four-cell rotation.

<sup>b</sup>SEM = Pooled standard error of the mean.

<sup>c</sup>1-5 scale; 1 = healthy; 5 = obese (Russel et al., 1969).

<sup>d</sup>1-5 scale; 1 = healthy; 5 = severely anemic (Bath et al., 2001).

<sup>e</sup>Analyzed using Chi-square procedure of SAS.

# Intake and digestibility of heat-damaged hay by Katahdin ewes

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## Story in Brief

The nutritional limitations of heat-damaged forage are well documented. However, ruminant animals have been observed to readily consume forages heated to the point of caramelization. Therefore, the objective was to determine intake and in vivo digestibility of bermudagrass hay having varied degrees of caramelization. Large round hay bales of predominantly bermudagrass with varying degrees of caramelization among and within bales were identified. Core samples were taken from specific locations within the bales to validate visual degree of heat-damage with chemical analyses. Hay from the bales was then separated into 3 levels of heat-damage based on visual color. Prior to feeding, hay was chopped using a bedding chopper to an approximate fiber length of one inch. Fifteen non-pregnant, non-lactating Katahdin ewes ( $147.2 \pm 2.91$  lb initial body weight) were stratified by weight within age and allocated randomly to 1 of the 3 treatments. Ewes were offered their respective hays for ad libitum consumption through a 10-day adaptation period followed by 5 days of total fecal collection. Dry matter intake and digestibility of dry matter and neutral- and acid-detergent fiber, as well as digestible dry matter and organic matter intake were greater ( $P < 0.05$ ) from hays with low and medium caramelization compared with the highly-caramelized hay. Organic matter digestibility differed ( $P < 0.05$ ) among all 3 hays. Therefore, when provided as the only dietary choice, intake and digestibility of severely caramelized hay may be reduced compared with that of non-caramelized hay.

## Introduction

Heat damage in forages is a result of high moisture at baling in combination with improper storage methods (McBeth et al., 2001). Respiration is directly related to moisture level at the time of storage and respiration losses constitute a significant dry matter loss in which soluble sugars are lost as carbon dioxide, water and heat (Rotz and Muck, 1994). Turner et al. (2002) reported that bermudagrass hay (20% or greater moisture at baling) increased in neutral-detergent fiber, acid-detergent fiber and acid-detergent lignin. This can be explained by preferential respiration of nonstructural carbohydrates in the microbial degradation of the forage (Coblentz et al., 1997). Likewise, heat-damaged forage was documented to be lower in both organic matter digestibility and apparent nitrogen absorption (McBeth et al., 2001). Ruminant animals though, were observed to preferentially consume forage that was heated to the point of caramelization. The objective was to determine intake and in vivo digestibility of bermudagrass hay having varied degrees of caramelization.

## Materials and Methods

All animal procedures used in this study were approved by the University of Arkansas Institutional Animal Care and Use Committee.

Six large-round bales of predominately bermudagrass [*Cynodon dactylon* (L.) Pers.] hay, grown at the Savoy Stocker Cattle Research Center at the University of Arkansas, were identified to have sustained varying levels of heat damage. Core samples were collected and analyzed for concentrations of acid-detergent insoluble nitrogen (ADIN) as an indication of the degree of heat damage. Once identified, hay was hand-sorted into three different levels of damage (LOW, MED, HIGH) based on visual and olfactory observations. Samples of each level of heat damage were collected for determination of species composition.

Fifteen non-pregnant, non-lactating Katahdin ewes (*Ovis aries*) from Lincoln University (Jefferson City, Mo.) were stratified by initial

body weight ( $147.2 \pm 2.91$  lb) and assigned randomly to one of the three treatments. Animals were housed at the University of Arkansas in 5-ft by 14-ft stalls fitted with smooth rubber flooring.

Prior to feeding, all treatment hays were chopped to an approximate length of 1 inch using a bedding chopper. Diets were offered for ad libitum consumption in two meals at 0800 and 1600 hours daily for a 10-day adaptation period. This was followed by 5 days of total fecal collections where feces were collected at 0800 and 1600 hours daily. Rejected feed was collected from feed bunks at 1600 hours daily. Water was offered for ad libitum consumption throughout the trial, and 0.4 ounces of a commercial trace mineral supplement was fed at 1600 hours daily. The trace mineral supplement (Ragland Feeds, Neosho, Mo.) contained not less than 9% calcium, 6% phosphorus, 35% salt, 1% magnesium, 1% potassium, 1% sulfur, 125 ppm cobalt, 150 ppm iodine, 5000 ppm iron, 10 ppm selenium, 140 ppm zinc, 160,000 IU/lb vitamin A, 40,000 IU/lb vitamin D3, and 150 IU/lb vitamin E.

Hay, rejected feed, and fecal samples were analyzed for dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), nitrogen (N) and fiber-bound N. Samples were also hand-sieved to determine particle size. Likewise, a representative sample of each of the composites (hay, orts) was taken, placed in a foam meat tray, wrapped in transparent film, and scanned using the Hunter MiniScan (Hunter Associates Laboratory, Inc., Reston, Va.) for color determination. Statistics were analyzed using the MIXED procedure of SAS (SAS Institute, Inc, Cary, N.C.) where animal served as the experimental unit.

## Results and Discussion

Treatments were similar in species composition with bermudagrass as the predominant forage present (Table 1). Dallisgrass (*Paspalum dilatatum* Poir.), goosegrass [*Eleusine indica* (L.) Gaertn.], johnsongrass [*Sorghum halepense* (L.) Pers.] and yellow foxtail [*Setaria pumila* (Poir.) Roem. and Schult. ssp. *pumila*] were also

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present. Organic matter (OM) and NDF concentrations decreased, but ADF concentrations increased ( $P < 0.05$ ) with increasing degree of caramelization. Our results are contradictory to the findings of others reporting heat-damaged hay research (Coblentz et. al., 1997; Rotz and Muck, 1994; Turner et. al., 2002), but these groups did not examine caramelized forage. Crude protein (CP) concentrations were similar among treatments, but acid-detergent insoluble nitrogen (ADIN) increased ( $P < 0.05$ ) with increasing degree of caramelization. Forages were darker, more red, and less yellow in color as the degree of caramelization increased. Also, the percentage of small particles increased with increasing heat damage or caramelization.

Dry matter intake (DMI), organic matter intake (OMI) and DM digestibility decreased ( $P < 0.05$ ) from HIGH compared with LOW and MED, and organic matter digestibility (OMD) decreased ( $P < 0.05$ ) with increasing heat damage (Table 2). This decrease in OMD is in agreement with the results reported in McBeth et. al. (2001), who saw a decrease from 58.6% with 5 heating-degree days to 54.0% with 401 heating-degree days. Digestible DMI and OMI were both lower ( $P < 0.05$ ) from HIGH compared with LOW and MED. Digestibility of NDF and ADF were also lower ( $P < 0.05$ ) from HIGH compared with LOW and MED. This decrease in NDF digestibility is more exaggerated than that reported by McBeth et. al. (2001), and the decrease in ADF digestibility is significant in this study where it was not in the previous work.

Rejected feed was darker (negative change in  $L^*$ ) for HIGH and lighter (positive change in  $L^*$ ) for LOW and MED compared with the original hay offered ( $P < 0.05$ ; Table 3). This was interpreted to mean that darker, more severely damaged portions of HIGH were rejected; whereas lighter, more stemmy portions of LOW and MED were rejected (increase in  $b^*$  value). Change in particles greater than 0.08 inches in the rejected feed compared with feed offered was not statistically different among treatments. Particles from 0.04 to 0.08 inches tended to increase in MED and HIGH rejected feed and decrease in LOW compared with the original hay offered ( $P < 0.10$ ). Particles from 0.01 to 0.04 inches increased ( $P < 0.05$ )

and particles less than 0.01 inches tended to increase ( $P < 0.10$ ) in HIGH compared with LOW and MED from the hay offered to the hay refused. This implies that the excessively-caramelized hay was more brittle, resulting in greater shattering into small particles that the sheep did not readily consume.

### Implications

Both intake and digestibility of caramelized forage is low by ewes when compared with undamaged and lightly damaged forages. This is contradictory to observations and testimonials of some producers. Apparent consumption of caramelized hay may be confused with greater shattering, giving the illusion of consumption. Based on the data collected in this study, combined with previous work on the issue of heat-damaged forage, we conclude that intake and digestibility of caramelized hay will not be sufficient to maintain ruminant animals.

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**Table 1. Characterization of treatment hays based on species composition and chemical properties.**

Item <sup>†</sup>	LOW <sup>‡</sup>	MED	HIGH
<i>Species composition (%)</i>			
Bermudagrass	88.1	83.1	90.7
Dallisgrass	0.3	0.4	0.0
Goosegrass	0.0	0.4	0.1
Johnsongrass	3.9	15.0	8.7
Yellow foxtail	7.7	1.0	0.3
Unidentified	0.0	0.0	0.2
<i>Chemical analysis</i>			
Dry matter (%)	91.5	91.8	93.2
Organic matter (%)	91.5	89.0	87.6
NDF (% DM)	73.8	70.6	57.6
ADF (% DM)	35.2	37.3	46.4
CP (% DM)	15.2	15.9	16.4
NDIN (% N)	62.5	67.8	62.8
ADIN (% N)	16.3	28.2	57.9
<i>Color values</i>			
L*	51.3	45.1	26.4
a*	4.8	6.1	7.7
b*	19.7	18.6	9.7
<i>Particle size<sup>§</sup></i>			
Large (> 0.16 inches)	45.0	39.6	5.0
0.16 inches	32.9	45.0	32.2
0.08 inches	16.8	9.0	31.0
0.04 inches	3.3	3.0	13.9
0.02 inches	1.5	2.2	10.1
0.01 inches	0.5	1.3	7.8

<sup>†</sup> NDF = neutral-detergent fiber; ADF = acid-detergent fiber; CP = crude protein; NDIN = neutral detergent insoluble nitrogen; ADIN = acid detergent insoluble nitrogen; L\* = lightness; a\* = redness; b\* = yellowness.

<sup>‡</sup> LOW = mild heat damage; MED = moderate heat damage; HIGH = severe caramelization.

<sup>§</sup> Expressed as percent of material passing through each screen.

**Table 2. Intake and digestibility of bermudagrass hay with varied degrees of caramelization by ewes.**

Item <sup>†</sup>	LOW <sup>‡</sup>	MED	HIGH	SEM <sup>§</sup>
Dry matter intake (% BW)	2.5 <sup>a</sup>	2.4 <sup>a</sup>	1.6 <sup>b</sup>	0.20
Organic matter intake (% BW)	2.2 <sup>a</sup>	2.1 <sup>a</sup>	1.4 <sup>b</sup>	0.17
Dry matter digestibility (%)	56.2 <sup>a</sup>	53.5 <sup>a</sup>	34.7 <sup>b</sup>	0.92
Organic matter digestibility (%)	58.6 <sup>a</sup>	55.4 <sup>b</sup>	36.7 <sup>c</sup>	0.99
Digestible DMI (% BW)	1.4 <sup>a</sup>	1.3 <sup>a</sup>	0.6 <sup>b</sup>	0.10
Digestible OMI (% BW)	1.3 <sup>a</sup>	1.2 <sup>a</sup>	0.5 <sup>b</sup>	0.08
NDF digestibility (%)	64.9 <sup>a</sup>	63.2 <sup>a</sup>	50.0 <sup>b</sup>	1.04
ADF digestibility (%)	64.4 <sup>a</sup>	63.6 <sup>a</sup>	29.9 <sup>b</sup>	1.64
CP digestibility (%)	53.3 <sup>a</sup>	50.3 <sup>a</sup>	6.5 <sup>b</sup>	1.84

<sup>a,b,c</sup> Means within a row differ ( $P < 0.05$ ).

<sup>†</sup> BW = body weight; DMI = dry matter intake; OMI = organic matter intake; NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein.

<sup>‡</sup> LOW = mild heat damage; MED = moderate heat damage; HIGH = severe caramelization.

<sup>§</sup> Pooled standard error of the mean.

**Table 3. Change in color and particle size between hay offered and hay refused by ewes offered bermudagrass hay with varied degrees of heat damage.**

Item <sup>†</sup>	LOW <sup>‡</sup>	MED	HIGH	SEM <sup>§</sup>	P-value <sup>¶</sup>
Color change (percentage units)					
L*	3.2 <sup>a</sup>	5.7 <sup>b</sup>	-2.4 <sup>c</sup>	0.75	< 0.0001
a*	-0.2 <sup>a</sup>	0.1 <sup>b</sup>	-0.2 <sup>a</sup>	0.09	0.0499
b*	0.8 <sup>a</sup>	1.4 <sup>a</sup>	-1.8 <sup>b</sup>	0.31	< 0.0001
Particle size change <sup>#</sup>					
Large	18.6	6.2	-2.8	7.34	0.1586
0.16 inches	-10.7	-15.2	-23.0	4.12	0.1427
0.08 inches	-7.6 <sup>d</sup>	8.4 <sup>f</sup>	1.4 <sup>e</sup>	4.71	0.0951
0.04 inches	-0.5 <sup>a</sup>	-0.1 <sup>a</sup>	8.8 <sup>b</sup>	2.62	0.0447
0.02 inches	0.0 <sup>a</sup>	0.3 <sup>a</sup>	8.3 <sup>b</sup>	1.99	0.0192
0.01 inches	0.1 <sup>d</sup>	0.5 <sup>d</sup>	7.3 <sup>e</sup>	2.36	0.0930

<sup>a,b,c</sup> Means within a row differ ( $P < 0.05$ ).

<sup>d,e,f</sup> Means within a row differ ( $P < 0.10$ ).

<sup>†</sup> L\* = lightness; a\* = redness; b\* = yellowness.

<sup>‡</sup> LOW = mild heat damage; MED = moderate heat damage; HIGH = severe caramelization.

<sup>§</sup> Pooled standard error of the mean.

<sup>¶</sup> P-values listed are for main effects of treatment.

<sup>#</sup> Expressed as percent of material passing through each screen.

# Instrumental color properties of ground beef processed from beef trimmings treated with peroxyacetic acid and /or organic acids

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## Story in Brief

The risk of ground beef products being contaminated with pathogens can be moderated by application of various antimicrobial decontamination methods on beef trimmings prior to grinding. However, antimicrobial interventions may cause negative impacts on ground beef color which in turn lowers consumer demand for these products. Therefore, the objective of this study was to evaluate the instrumental color properties of ground beef processed from beef trimmings treated with peroxyacetic acid alone or followed by organic acids antimicrobial interventions prior to grinding. Inoculated (*E. coli* O157:H7 and non-STEC O157:H7 and *Salmonella* spp. cocktail mixture at 10<sup>5</sup> Colony-Forming Units (CFU)/g beef trimmings were treated (3 lb/treatment/replicate, 2 replicates) with conventional spray application of 0.02% peroxyacetic acid as a single intervention or in combined with conventional and electrostatic spray application of malic, pyruvic, octanoic acid at 3%, saturated solution of fumaric acid or deionized water (W). Subsequently, trimmings were ground twice and overwrapped-packaged (0.5 lb/sample) using plastic foam trays with absorbent pads and polyvinyl chloride film. The ground beef packages were displayed under simulated retail conditions (39 °F) and the color was measured on days 0, 1, 2, 3 and 7 days using a Hunter-Lab MiniScan XE Spectrocolorimeter. Findings from this study suggest that use of peroxyacetic acid as a single or multiple hurdle approach with malic, pyruvic, octanoic and fumaric acid on beef trimmings had little or no adverse color changes ( $P > 0.05$ ) compared to the untreated inoculated control ground beef samples. Additionally electrostatic spray application of pyruvic and fumaric treatments showed enhanced ( $P < 0.05$ ) redness in ground beef compared to the control sample on day 0 of display. Therefore, electrostatic application of organic acids may open new cost conscious prospects to enhance product safety without forfeiting the quality of the product.

## Introduction

Although acetic, citric and lactic acids are widely accepted as effective antimicrobial interventions in meat decontamination, it would be worth considering other food grade organic acids in such applications to reduce pathogenic bacteria populations in ground beef. Scientific evidence supports that a multiple chemical hurdle approach may obtain greater reduction of microbial populations than using a single intervention (Pohlman et al., 2002). Peroxyacetic acid has also been found to be effective against a wide range of pathogenic and spoilage organisms in meat (Quilo et al., 2010). Ground beef consumers not only demand product safety but also look for appearance of the product. Therefore, ground beef color is one of the main factors determining the consumers purchasing decisions as they discriminate against discolored meat products. Consequently, the objective of this study was to evaluate the impact of a multiple hurdle decontamination approach using peroxyacetic acid and octanoic acid, pyruvic acid, malic acid and fumaric acid on beef trimming prior to grinding on ground beef instrumental color properties.

## Materials and Methods

**Sample Preparation.** Frozen beef trimmings (80 lean meat/20 fat; 90 lb) obtained from Cargill Meat Solutions (Plainview, Texas) were thawed and divided equally and inoculated with a cocktail mixture (39 °F) of *E. coli* O157:H7, O26, O103, O111, O121, O45, and O145 and *Salmonella* Typhimurium DT 104 *Salmonella* Newport MDR-AmpC at 10<sup>5</sup> CFU/g (Pohlman et al., 2002). After leaving overnight at 4 °C for further bacterial attachment, the inoculated beef

trimmings (3 lb/treatment/replicate) were arranged on stainless steel trays for treatment application. As per manufacturer's recommendations, peroxyacetic acid treatment was applied using only a conventional spray system. Each side of the beef trimmings (3 lb/treatment/replicate) were first treated with conventional spray (~46 ml/lb) applications of 0.02% peroxyacetic acid (PA; Blitz<sup>®</sup>, FMC Corporation, Philadelphia, Pa.) as a single intervention or followed by conventional (CS) or electrostatic spray (ES; Electrostatic Spraying Systems, Inc. Watkinsville, Ga.) applications (~27 ml/lb) of 3% malic acid (PM; Sigma Aldrich St. Louis Mo.), 3% pyruvic acid (PP; Sigma Aldrich St. Louis Mo.), 3% octanoic acid (PO; Sigma Aldrich St. Louis Mo.), and saturated solution of fumaric acid (PF; Sigma Aldrich St. Louis Mo.). The PA-treated samples were allowed to drip for 3 min prior to and after assigned second antimicrobial applications (2 replicates/treatment). Each treatment was repeated two times. Untreated inoculated samples (CON) and inoculated beef trimmings treated with the conventional and electrostatic spray applications of de-ionized water (W) were retained as controls. All treated and untreated inoculated beef trimmings were ground twice and 0.5 lb of individual samples were placed on plastic foam trays and over wrapped with polyvinyl chloride film (O<sub>2</sub> transmission rate = 14,000 cc/mm<sup>2</sup>/24 h/1 atm; Koch Supplies, Inc., Kansas City, Mo.). The packages were stored under simulated retail conditions (39 °F, under 1,630 lux of deluxe warm white fluorescent lighting; Phillips Inc., Somerset, N.J.).

**Instrumental Color.** Following standardization of the Spectrocolorimeter (Hunter-Lab MiniScan XE Spectrocolorimeter, Model 4500L; Hunter Associates Laboratory, Reston, W. Va.) using white tile, black tile and working standards (Pohlman et al., 2002), instrumental color of samples were measured (n = 3/treatment) on day 0,

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1, 2, 3 and 7 of display. The ground beef samples were evaluated for CIE  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) color values using Illuminant A/10° observer. A reflectance measurement in the 580 to 630 nm visible spectrum was also acquired. The hue angle (hue or color of ground beef), saturation index (which describe the brightness or vividness of color) and reflectance ratio (proportion of oxymyoglobin of the myoglobin pigment) were calculated as  $(\arctan(b^*/a^*))$ ,  $((a^{*2} + b^{*2})^{0.5})$ , and  $(630/580 \text{ nm})$ , respectively.

**Analysis of Data.** The data 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 PDIF option of SAS.

## Results and Discussion

The ground beef samples processed from PA, W, PM and PF had similar lightness ( $L^*$ ) ( $P > 0.05$ ) to CON on days 0, 1, 3 and 7 of display regardless of the treatment application method (Table 1). Ground beef from beef trimmings treated with PP by both application methods had similar lightness to the CON on days 0, 3 and 7 of display. In contrast, the ground beef processed from PO treatment applied by the ES method was significantly ( $P < 0.05$ ) lighter compared to the control through the entire duration of display. Excluding ES applications of PP and PF treatments, all the treatments, despite application method, had similar ( $P > 0.05$ ) redness ( $a^*$ ) to CON on day 0 of display (Table 2). However, ground beef from PP and PF applied with ES showed higher redness ( $P < 0.05$ ) values compared to the control on day 0 of display. All the treatments applied through CS and ES methods, except ES application of malic acid on day 7 of display, maintained a similar redness to control during days 1 through 7 of display. The treatment and application method did not show an interaction effect on ground beef yellowness on days 0 through 7 of display with all treatments being similar in yellowness to the control and each other (Table 3). The hue angles of ground beef from all treatments showed no difference ( $P > 0.05$ ) on days 0 and 7 of display (Table 4). On day 1 of display, only W treatment by both application methods and PO by ES method showed a similar hue angle ( $P > 0.05$ ) to the control sample. All the treatments except CS application of PP, irrespective of application method, showed similar hue angle ( $P > 0.05$ ) to the control ground beef on days 2 and 4 of display. All treated samples possessed a saturation index similar ( $P > 0.05$ ) to control throughout the display time except ES applications of PF on day 0 of display (Table 5). Therefore, with the exception of PF applied by ES on day 0 of display, all treatments were as vivid in color throughout display as the control. Additionally, all treatments had similar reflectance ratio (estimated oxymyoglobin content)

compared to CON on days 0, 3 and 7 of display (Table 6). Hence, antimicrobial or application method had little impact on myoglobin state, keeping similar oxymyoglobin content as the control. Even though it is assumed that organic acid potentially could accelerate the oxidation of myoglobin causing undesirable quality attributes, previous research recognizes that the extent of treatment variability is responsible for such changes (Smulders and Greer, 1998). According to Quilo et al. (2009) application of 0.02% peroxyacetic as a single antimicrobial intervention on beef trimmings prior to grinding resulted in improving redness in bulk ground beef or patties. Correspondingly, our treated samples, on most occasions, showed similar or enhanced redness to the untreated control ground beef. The less antimicrobial usage and less run off waste highlight the efficiency of electrostatic spray application in organic acid meat decontamination interventions.

## Implications

Peroxyacetic acid alone or followed by conventional or electrostatic spray application of malic, pyruvic, octanoic or fumaric acid on beef trimmings had no or little interference on ground beef instrumental color. Further, the electrostatic application of organic acid may establish a cost-effective antimicrobial decontamination intervention in enhancing ground beef product safety without impairing the important quality attributes such as ground beef color.

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**Table 1. Effects of antimicrobial treatment, application method and day of display on ground beef lightness ( $L^*$ ) during simulated retail display storage at 4 °C.**

Treatment <sup>‡</sup>	Application Method <sup>§</sup>	Lightness ( $L^*$ ) <sup>†</sup>				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	51.08 <sup>C</sup>	50.45 <sup>C</sup>	48.59 <sup>C</sup>	46.98 <sup>B</sup>	46.42 <sup>B</sup>
PA	CS	54.45 <sup>abc</sup>	54.84 <sup>abc</sup>	53.74 <sup>abc</sup>	51.11 <sup>ab</sup>	50.25 <sup>ab</sup>
W	CS	53.61 <sup>abc</sup>	53.83 <sup>bc</sup>	51.92 <sup>abc</sup>	51.31 <sup>ab</sup>	49.86 <sup>ab</sup>
W	ES	53.53 <sup>abc</sup>	52.48 <sup>bc</sup>	49.46 <sup>bc</sup>	48.03 <sup>b</sup>	47.05 <sup>ab</sup>
PM	CS	54.55 <sup>abc</sup>	55.86 <sup>abc</sup>	55.23 <sup>ab</sup>	52.49 <sup>ab</sup>	50.43 <sup>ab</sup>
PM	ES	54.78 <sup>abc</sup>	55.14 <sup>abc</sup>	50.91 <sup>abc</sup>	50.44 <sup>ab</sup>	51.13 <sup>ab</sup>
PP	CS	55.59 <sup>abc</sup>	56.36 <sup>ab</sup>	56.38 <sup>a</sup>	53.84 <sup>ab</sup>	51.24 <sup>ab</sup>
PP	ES	53.79 <sup>abc</sup>	54.56 <sup>abc</sup>	51.05 <sup>abc</sup>	49.65 <sup>ab</sup>	48.30 <sup>ab</sup>
PO	CS	56.73 <sup>ab</sup>	56.53 <sup>ab</sup>	54.11 <sup>abc</sup>	53.07 <sup>ab</sup>	51.49 <sup>ab</sup>
PO	ES	58.45 <sup>a</sup>	59.56 <sup>a</sup>	56.19 <sup>a</sup>	56.34 <sup>a</sup>	54.28 <sup>a</sup>
PF	CS	52.78 <sup>bc</sup>	53.48 <sup>C</sup>	50.95 <sup>abc</sup>	48.78 <sup>ab</sup>	47.60 <sup>ab</sup>
PF	ES	52.68 <sup>bc</sup>	54.35 <sup>abc</sup>	51.85 <sup>abc</sup>	48.94 <sup>ab</sup>	48.06 <sup>ab</sup>
	SE	±0.97	±0.98	±1.17	±1.43	±1.39

<sup>a-c</sup> Least squares means within a column with different superscripts differed significantly ( $P < 0.05$ ).

<sup>†</sup> Lightness ( $L^*$ ) reported as least squares means along with  $\pm$  standard error.

<sup>‡</sup> Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

<sup>§</sup> Application methods: CS = conventional spray application, ES = electrostatic spray application.

**Table 2. Effects of antimicrobial treatment, application method and day of display on ground beef redness ( $a^*$ ) during simulated retail display storage at 4 °C.**

Treatment <sup>‡</sup>	Application Method <sup>§</sup>	Redness ( $a^*$ ) <sup>†</sup>				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	13.97 <sup>C</sup>	13.09	18.40 <sup>abc</sup>	20.30	20.21 <sup>a</sup>
PA	CS	15.59 <sup>abc</sup>	9.33	12.36 <sup>abc</sup>	17.78	18.16 <sup>a</sup>
W	CS	15.75 <sup>abc</sup>	11.17	18.68 <sup>ab</sup>	19.93	17.92 <sup>ab</sup>
W	ES	17.18 <sup>abc</sup>	12.16	19.97 <sup>a</sup>	19.60	17.81 <sup>ab</sup>
PM	CS	16.09 <sup>abc</sup>	8.95	10.12 <sup>bc</sup>	17.02	18.04 <sup>ab</sup>
PM	ES	17.19 <sup>abc</sup>	8.71	15.90 <sup>abc</sup>	16.12	14.62 <sup>b</sup>
PP	CS	14.56 <sup>bc</sup>	9.85	9.37 <sup>C</sup>	14.29	17.99 <sup>ab</sup>
PP	ES	18.06 <sup>ab</sup>	8.97	15.04 <sup>abc</sup>	19.21	18.02 <sup>ab</sup>
PO	CS	16.13 <sup>abc</sup>	9.45	16.93 <sup>abc</sup>	19.49	19.80 <sup>ab</sup>
PO	ES	15.12 <sup>abc</sup>	10.08	17.43 <sup>abc</sup>	18.80	18.65 <sup>ab</sup>
PF	CS	17.89 <sup>abc</sup>	9.59	15.82 <sup>abc</sup>	20.04	19.97 <sup>ab</sup>
PF	ES	18.98 <sup>a</sup>	9.56	14.10 <sup>abc</sup>	17.93	18.85 <sup>ab</sup>
	SE	±0.70	±0.86	±1.63	±1.43	±0.97

<sup>a-c</sup> Least squares means within a column with different superscripts differed significantly ( $P < 0.05$ ).

<sup>†</sup> Redness ( $a^*$ ) reported as least squares means along with  $\pm$  standard error.

<sup>‡</sup> Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

<sup>§</sup> Application methods: CS = conventional spray application, ES = electrostatic spray application.

**Table 3. Effects of antimicrobial treatment, application method and day of display on ground beef yellowness ( $b^*$ ) during simulated retail display storage at 4 °C.**

Treatment <sup>‡</sup>	Application Method <sup>§</sup>	Yellowness ( $b^*$ ) <sup>†</sup>				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	16.34	15.36	15.03	15.37	14.27
PA	CS	16.88	16.40	15.06	16.19	14.25
W	CS	16.87	16.08	17.36	16.57	13.64
W	ES	16.76	15.60	16.55	15.17	12.51
PM	CS	17.25	15.89	16.73	17.55	14.89
PM	ES	17.31	15.50	14.97	15.09	12.41
PP	CS	17.39	17.24	16.77	17.42	15.21
PP	ES	17.31	15.97	16.42	16.80	13.88
PO	CS	18.00	16.55	17.31	17.62	15.73
PO	ES	16.52	15.76	16.56	17.23	15.15
PF	CS	18.11	16.77	16.99	17.59	14.95
PF	ES	18.55	16.14	16.41	15.89	14.35
	SE	0.50	0.49	0.87	0.45	0.66

<sup>†</sup> Yellowness ( $b^*$ ) reported as least squares means along with  $\pm$  standard error.

<sup>‡</sup> Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

<sup>§</sup> Application methods: CS = conventional spray application, ES = electrostatic spray application.

**Table 4. Effects of antimicrobial treatment, application method and day of display on ground beef hue angle during simulated retail display storage at 4 °C.**

Treatment <sup>‡</sup>	Application Method <sup>§</sup>	Hue angle <sup>†</sup>				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	49.48	49.56 <sup>b</sup>	39.32 <sup>c</sup>	37.19 <sup>b</sup>	35.27
PA	CS	47.27	60.33 <sup>a</sup>	50.71 <sup>abc</sup>	42.33 <sup>ab</sup>	37.98
W	CS	46.97	55.45 <sup>ab</sup>	42.95 <sup>c</sup>	39.73 <sup>b</sup>	37.33
W	ES	44.26	52.36 <sup>ab</sup>	39.72 <sup>c</sup>	37.84 <sup>b</sup>	35.11
PM	CS	47.07	60.65 <sup>a</sup>	58.81 <sup>abc</sup>	45.99 <sup>ab</sup>	39.57
PM	ES	45.18	60.65 <sup>a</sup>	43.24 <sup>c</sup>	43.17 <sup>ab</sup>	40.35
PP	CS	50.09	60.23 <sup>a</sup>	60.79 <sup>a</sup>	51.39 <sup>a</sup>	40.14
PP	ES	43.81	60.67 <sup>a</sup>	47.63 <sup>bc</sup>	41.17 <sup>ab</sup>	37.61
PO	CS	48.14	60.25 <sup>a</sup>	46.07 <sup>c</sup>	42.09 <sup>ab</sup>	38.45
PO	ES	47.50	57.47 <sup>ab</sup>	43.52 <sup>c</sup>	42.51 <sup>ab</sup>	39.08
PF	CS	45.37	60.26 <sup>a</sup>	47.50 <sup>bc</sup>	41.29 <sup>ab</sup>	36.79
PF	ES	44.35	59.33 <sup>a</sup>	49.44 <sup>abc</sup>	41.55 <sup>ab</sup>	37.30
	SE	1.17	1.62	2.09	1.99	1.12

<sup>a-c</sup> Least squares means within a column with different superscripts differed significantly ( $P < 0.05$ ).

<sup>†</sup> Hue angle [ $\tan^{-1}(b^*/a^*)$ ] reported as least squares means along with  $\pm$  standard error.

<sup>‡</sup> Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

<sup>§</sup> Application methods: CS = conventional spray application, ES = electrostatic spray application.

**Table 5. Effects of antimicrobial treatment, application method and day of display on ground beef saturation index during simulated retail display storage at 4 °C.**

Treatment <sup>‡</sup>	Application Method	Saturation index <sup>†</sup>				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	21.51 <sup>b</sup>	20.21	23.77	25.48	24.76 <sup>ab</sup>
PA	CS	23.00 <sup>ab</sup>	18.88	19.51	24.06	23.14 <sup>ab</sup>
W	CS	23.09 <sup>ab</sup>	19.61	25.52	25.92	22.53 <sup>ab</sup>
W	ES	24.02 <sup>ab</sup>	19.82	25.94	24.79	21.77 <sup>ab</sup>
PM	CS	23.60 <sup>ab</sup>	18.24	19.56	24.48	23.40 <sup>ab</sup>
PM	ES	24.40 <sup>ab</sup>	17.78	21.84	22.09	19.18 <sup>b</sup>
PP	CS	22.68 <sup>ab</sup>	19.86	19.21	22.65	23.56 <sup>ab</sup>
PP	ES	25.04 <sup>ab</sup>	18.32	22.28	25.56	22.75 <sup>ab</sup>
PO	CS	24.18 <sup>ab</sup>	19.06	24.27	26.29	25.29 <sup>a</sup>
PO	ES	22.41 <sup>b</sup>	18.72	24.05	25.51	24.03 <sup>ab</sup>
PF	CS	25.46 <sup>ab</sup>	19.31	23.30	26.68	24.95 <sup>ab</sup>
PF	ES	26.55 <sup>a</sup>	18.76	21.65	23.97	23.70 <sup>ab</sup>
	SE	0.72	0.82	1.64	1.01	1.08

<sup>a-b</sup> Least squares means within a column with different superscripts differed significantly ( $P < 0.05$ ).

<sup>†</sup> Saturation index ( $[(a^*2+b^*)^{0.5}]$ ) reported as least squares means along with  $\pm$  standard error.

<sup>‡</sup> Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

<sup>§</sup> Application methods: CS = conventional spray application, ES = electrostatic spray application.

**Table 6. Effects of antimicrobial treatment, application method and day of display on ground beef reflectance ratio during simulated retail display storage at 4 °C.**

Treatment <sup>‡</sup>	Application Method <sup>§</sup>	Reflectance ratio <sup>†</sup>				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	2.82 <sup>abc</sup>	1.38 <sup>a</sup>	2.20 <sup>ab</sup>	2.53	2.37
PA	CS	2.83 <sup>abc</sup>	1.04 <sup>b</sup>	1.45 <sup>bcd</sup>	2.21	2.59
W	CS	2.86 <sup>ab</sup>	1.21 <sup>ab</sup>	2.12 <sup>ab</sup>	2.46	2.36
W	ES	3.18 <sup>a</sup>	1.29 <sup>ab</sup>	2.41 <sup>a</sup>	2.55	2.68
PM	CS	2.84 <sup>abc</sup>	1.05 <sup>ab</sup>	1.11 <sup>d</sup>	2.08	2.38
PM	ES	2.92 <sup>ab</sup>	1.05 <sup>ab</sup>	1.89 <sup>abc</sup>	2.19	2.13
PP	CS	2.58 <sup>bc</sup>	1.09 <sup>ab</sup>	1.29 <sup>cd</sup>	1.81	2.37
PP	ES	3.14 <sup>ab</sup>	1.07 <sup>ab</sup>	1.86 <sup>abcd</sup>	2.44	2.48
PO	CS	2.78 <sup>abc</sup>	1.07 <sup>ab</sup>	1.97 <sup>abc</sup>	2.33	2.35
PO	ES	2.28 <sup>c</sup>	1.34 <sup>ab</sup>	1.96 <sup>abc</sup>	2.33	2.68
PF	CS	3.17 <sup>a</sup>	1.02 <sup>b</sup>	1.77 <sup>abcd</sup>	2.33	2.42
PF	ES	3.34 <sup>a</sup>	1.06 <sup>ab</sup>	1.65 <sup>bcd</sup>	2.26	2.21
	SE	0.10	0.06	1.33	0.18	0.22

<sup>a-c</sup> Least squares means within a column with different superscripts differed significantly ( $P < 0.05$ ).

<sup>†</sup> Reflectance ratio (580/630 nm) reported as least squares means along with  $\pm$  standard error.

<sup>‡</sup> Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

<sup>§</sup> Application methods: CS = conventional spray application, ES = electrostatic spray application.



# Microbial characteristics of ground beef processed from beef trimmings decontaminated by peroxyacetic acid alone or followed by organic acids interventions

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## Story in Brief

Recent large numbers of ground beef recalls due to presence of possible *Escherichia coli* or *Salmonella* contaminations warrants novel and efficient decontamination applications to enhance ground beef microbial safety. The objective of this study was to evaluate the effectiveness of antimicrobial interventions using peroxyacetic acid followed by organic acids on beef trimmings prior to grinding on ground beef microbial populations. Beef trimmings were inoculated using a cocktail mixture of *Escherichia coli* O157:H7, non- O157:H7 shiga toxin-producing *E. coli* and *Salmonella* spp. (10<sup>5</sup> CFU/g). Inoculated trimmings (3 lb/treatment/replicate, 2 replicates) were treated with conventional spray application of 0.02% peroxyacetic acid alone or followed by conventional or electrostatic spray application of octanoic acid, pyruvic acid or malic acid at 3% concentration, saturated solution of fumaric acid or deionized water. Subsequently, trimmings were ground twice and were placed on plastic foam trays with absorbent pads and overwrapped with polyvinyl chloride film and sampled on days 0, 1, 2, 3 and 7 days of simulated display for microbial counts and instrument color properties. Findings from this study suggest that peroxyacetic acid as a single or multiple hurdle approach with malic, pyruvic, octanoic and fumaric acid on beef trimmings may be effective in reducing *E. coli* O157:H7 as well as Non-STEC serotypes and *Salmonella* up to day 2 of display. The results also indicate that electrostatic application of some organic acids may have similar or greater efficiency in controlling ground beef microbial populations compared to the conventional application of the same acid providing more economical and waste manageable decontamination approach.

## Introduction

*Escherichia coli* and *Salmonella* are important foodborne pathogens associated with ground beef and responsible for a large number of foodborne illness cases in the United States (Mead et al., 1995). Further, non-O157 Shiga toxin strains have emerged and especially O26, O45, O103, O111, O121, and O145 have been associated with large outbreaks of human disease (Nataro and Kaper, 1998). Additionally, the U.S. Department of Agriculture Food Safety and Inspection Services highlights the need for further research to reduce *Salmonella* Typhimurium definitive type 104 (DT104) in meat as it can exhibit a multi-drug resistance pattern to many antibiotics along with foodborne illness challenges. Organic acid aqueous (1-3%) spray or dip surface treatments proven to be effective in reducing microbial populations in meat carcass processing (Dickson and Anderson, 1992). Peroxyacetic acid, an equilibrium mixture of hydrogen peroxide and peracetic acid, showed efficient pathogenic and spoilage bacterial population reductions in meat (Pohlman et al., 2009). Therefore, our objective was to determine the effect of peroxyacetic acid and under-utilized organic acids on beef trimmings to control pathogenic bacteria in ground beef. Further the efficiencies of electrostatic spray and conventional spray application methods were compared to develop cost-conscious intervention approaches.

## Materials and Methods

**Inoculum Preparation.** A bacterial cocktail containing 10<sup>5</sup> log CFU *E. coli* O157:H7, O26, O103, O111, O121, O45 and O145 (EC), *Salmonella* Typhimurium DT 104, and *S. Newport* (SA) were prepared from frozen (-112 °F) pure cultures. To make the

cocktail, frozen samples were thawed and 0.1 ml of each strain was inoculated into 10 ml individual aliquots of Brain Heart Infusion solution (BHI; Difco Laboratories Becton Dickinson and Company, Sparks Md.). Then the inoculated tubes were incubated at 98 °F for 18 hours non-shaking (VWR Model 3015 incubator, VWR Scientific, West Chester, Pa.). Following incubation, the tubes were centrifuged at 3500 g for 20 min at 98 °F (Beckman GS-6 series, Fullerton, Calif.). Next the liquid supernatant was discarded and the bacterial pellets were re-suspended with buffered peptone water (BPW; Difco Laboratories, Becton Dickinson and Company, Sparks Md.). Finally, equal volumes of each strain was mixed together and further diluted with BPW to achieve 10<sup>5</sup> CFU/ml nine-strain cocktail mixtures of EC and SA and stored at 39 °F until further use.

**Meat Inoculation.** Frozen beef trimmings (80/20; 90 lb) obtained from Cargill Meat Solutions (Plainview, Texas) were thawed and divided into sterile biohazard bags. The cocktail mixture of *E. coli* (EC) and *Salmonella* (SA) at 10<sup>5</sup> CFU/g was incorporated into trimming portions and mixed to acquire an even inoculum distribution on beef trimmings. Then the inoculated trimmings were separated into 3 lb batches (replicate/treatment) and left overnight at 39 °F to allow bacterial attachment.

**Treatment Application.** As per manufacturer's recommendations, peroxyacetic acid treatment application was confined to a conventional spray system. The beef trimmings (3 lb/treatment/replicate), prearranged on stainless steel trays, were first treated with conventional spray (~46 ml/lb) applications of 0.02% peroxyacetic acid (PA; Blitz®, FMC Corporation, Philadelphia, Pa.) as a single intervention or followed by conventional (CS) or electrostatic spray (ES; Electrostatic Spraying Systems, Inc. Watkinsville, Ga.) applications (~27 ml/lb) of 3% malic acid (PM; Sigma Aldrich St. Louis Mo.), 3% pyruvic acid (PP; Sigma Aldrich St. Louis Mo.), 3% octanoic

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acid (PO; Sigma Aldrich St. Louis Mo.), and saturated solution of fumaric (PF; Sigma Aldrich St. Louis Mo.). The PA-treated samples were allowed to drip for 3 min prior to and after assigned second antimicrobial applications (2 replicates/treatment). Inoculated beef trimmings were also treated with the conventional and electrostatic spray applications of de-ionized water (W) at the same rates used in antimicrobial applications and dripped for 3 min. Each treatment was repeated two times. Untreated inoculated samples were retained as a control (CON).

**Meat Processing.** Following treatment application phase, all treated and untreated inoculated (CON) beef trimmings were ground (American Eagle Model: AEG-12N, #14 (1/8 inches) chopper plate) twice and 0.5 lb of individual samples were placed on plastic foam trays and over wrapped with polyvinyl chloride film. The ground beef packages were displayed under retail condition (39 °C; 1,630 lx of deluxe warm white fluorescent lighting; Phillips Inc., Somerset, N.J.) and sampled on day 0, 1, 2, 3, and 7 day of display for microbial analysis and CIE  $L^*$ ,  $a^*$  and  $b^*$  measurements.

**Microbial Enumeration.** A sample of 25 g was aseptically removed from each ground beef sample and placed in sterile whirl-pack bags (Nasco, Ft Atkinson, Wis.) separately. These samples were incorporated with 225 ml of 0.1% buffered peptone water and homogenized for 2 minutes at normal speed (Model 400 Lab Stomacher; Seward, London, UK). Subsequently, serial 10-fold dilutions were made and duplicate spread plating (SA 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 carried out. The EC, APC and ST counts were read after 48 h incubation at 98 °F (VWR Model 5015, or Model 3015, VWR Scientific, West Chester, Pa.); whereas coliform plates were read after 24 h.

**Statistical Analysis.** The experimental design included treatments, application methods and 5 display days (0, 1, 2, 3 and 7). Treatments were blocked by replicate and then analyzed for the main effects of antimicrobial treatment, day of display and treatment by day interactions. Least square means for significant main effects were identified using the LSMEANS PDIF option of SAS (version 9.2, SAS Institute Inc., Cary, N.C.).

## Results and Discussion

**Coliform.** All the treatments showed a significant reduction ( $P < 0.05$ ) in ground beef coliform counts compared to the inoculated control on day 0 (Table 1). However, the PA, W and PM treatments by CS application, PM, PP, and PF treatments by ES application showed more than 1 log reduction ( $P < 0.05$ ) of ground beef coliforms (CO) compared to the other treatments by both CS and ES methods. Considering all the treatments and application methods, PM (1.8 log) and PP (1.75 log) by CS method were most efficient in CO log reduction ( $P < 0.05$ ) for day 1 of display. Conversely, PA, W, and PF by ES, PP by CS and treatments exceeded ( $P < 0.05$ ) the other treatments in controlling CO counts with more than 1 log reduction on day 2 of display. None of the treatments showed significant CO reductions ( $P > 0.05$ ) on day 7 of display. The PP treatment applied through ES system outperformed the CS application in controlling ground beef CO population on day 0 of display. On the other hand, there was no significant difference ( $P > 0.05$ ) between CS vs. ES methods of W, PM, PO and PF treatments in ground beef CO reduction on day 0 of display. Therefore, ES application of these antimicrobials was able to achieve similar CO reduction as CS, but using much less antimicrobial.

**Escherichia Coli.** Although all the treatments resulted less in *Escherichia coli* (EC) population compared to the control (CON) sample, ground beef processed with PA, W, and PP, treatments applied through the CS method achieved over 1 log reduction on day 0 of display (Table 2). The PM, and PP treatments applied through the CS method, showed the lowest ( $P < 0.05$ ) EC count compared to all the treatments on day 1 of display with up to more than 1.9 log reduction. However, PO and PF treatments by both application methods together with PM and PP applied through the ES system also possessed significantly lower ( $P < 0.05$ ) EC counts compared to the control with 1 or more log reduction on day 1 of display. While PA along with ES application of PP, PO, and PF showed over 1 log reduction, CS application of PM and PP treatments accounted for more than 2 log reduction in EC counts on day 2 of display. The CS and ES application methods showed no difference ( $P > 0.05$ ) in reducing EC counts for PP, PO or PF treatments on day 3 of display. In contrast, PP and PM treatments were more efficient ( $P < 0.05$ ) in CS application method compared to the ES method in reducing EC populations in ground beef on day 1 and 2 of display, respectively.

**Aerobic Plate Counts.** The PA and PO-treated ground beef through CS application lead by CS application of PP, showed the lowest ( $P < 0.05$ ) aerobic plate count (APC) on day 0 of display (Table 1.3). On day 1 of display, CS application of PM treatment along with PP and PF treatments applied by both methods obtained over 2 log reduction of ground beef APC. The PP-treated ground beef through CS application had the lowest ( $P < 0.05$ ) APC with 2.05 log reduction on day 2 of display. Although ground beef from PP applied by ES had higher ( $P < 0.05$ ) APC compared to CS, it accounted for over 1 log reduction of APC on day 2 of display. Further, the PP by ES treatment was able to maintain a 1.02 log reduction of APC on day 3 of display. Both ES and CS treatment application methods of PO and PF treatments showed a similar ( $P > 0.05$ ) efficiency in controlling ground beef APC on day 1 of display. All treatments regardless of application method were effective for reducing ( $P < 0.05$ ) APC by day 7 of display.

**Salmonella.** Beef trimmings treated with PA, ES application of PM, PP, and PF along with CS application of PP and PO reduced ( $P < 0.05$ ) *Salmonella* (ST) population with above 1 log reduction on day 0 of display (Table 4). These treatments along with W and PO treatments through ES and CS application of PM and PF had significantly lower ( $P < 0.05$ ) *Salmonella* populations compared to CON, and CS application of W on days 1 and 2 of display. The CS application of PP indicated the lowest ( $P < 0.05$ ) ground beef *Salmonella* count on day 7. The ES applications of PM and PP had greater ( $P < 0.05$ ) ST count reductions compared to CS applications of same organic acids on day 0 of display. However, PM, PP, and PF treatments applied by both methods showed similar ( $P > 0.05$ ) ST reduction on days 1 and 2 of display. By day 1 of display, all treatments (with exception of water applied by CS) and application methods were effective ( $P < 0.05$ ) for reducing *Salmonella* counts.

Quilo et al. (2009) reported that peroxyacetic acid as a single antimicrobial intervention on beef trimmings could reduce *Escherichia coli* and nalidixic acid resistant *Salmonella* Typhimurium in ground beef. In agreement, our results signified the competence of peroxyacetic acid with 1.4 log reduction in cumulative populations of *E. coli* O157:H7 and six serogroups of non-O157:H7 and 1.7 log *Salmonella* population (*Salmonella* Typhimurium DT 104 and *Salmonella* Newport MDR-AmpC) when applied as a single intervention on beef trimmings. Additionally, even though application of multiple interventions were expected to enhance microbial reductions, peroxyacetic acid alone achieved lower reduction in

*E. coli* and *Salmonella* populations on day 0 of display than did multiple treatment combinations. However, this trend was changed on day 1 of display and some multiple treatment combinations prompted higher effectiveness in reducing *E. coli* and *Salmonella* populations. Among all the organic acids tested, fumaric acid had very poor water solubility. However, it showed a comparable antimicrobial effect to other organic acids tested. Our results also showed electrostatic application of some organic acids may have similar or greater efficiency in controlling ground beef microbial populations compared to the conventional spray application of the same acid.

### Implications

Peroxyacetic acid alone or followed by conventional or electrostatic spray application of malic, pyruvic, octanoic or fumaric acid on beef trimmings may be effective in reducing *E. coli* O157:H7 as well as non-O157 shiga toxin producing serotypes and *Salmonella* through 2 days of display. The electrostatic spray application of organic acid established a cost-conscious treatment application with less antimicrobial usage as well as improved waste management. Therefore, the outcome of this study opens new avenues for cost-effective utilization of natural organic acids in more efficient decontamination interventions in ground beef production lines to reduce pathogens of recent concerns.

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**Table 1. Effects of antimicrobial treatment, application method and day of display against coliform population in ground beef during simulated retail display storage at 4 °C.**

Treatment <sup>‡</sup>	Application Method <sup>§</sup>	Coliform count (log CFU/g) <sup>†</sup>				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	5.17 <sup>a</sup>	5.51 <sup>a</sup>	5.74 <sup>a</sup>	6.02 <sup>b</sup>	7.09
PA	CS	3.74 <sup>d</sup>	4.62 <sup>bc</sup>	4.56 <sup>d</sup>	5.98 <sup>b</sup>	6.79
W	CS	4.12 <sup>bc</sup>	5.67 <sup>a</sup>	5.73 <sup>a</sup>	6.27 <sup>a</sup>	7.09
W	ES	4.39 <sup>b</sup>	5.47 <sup>a</sup>	4.56 <sup>d</sup>	5.98 <sup>b</sup>	6.86
PM	CS	3.94 <sup>cd</sup>	3.71 <sup>d</sup>	4.91 <sup>cd</sup>	5.26 <sup>f</sup>	6.89
PM	ES	4.16 <sup>bc</sup>	4.97 <sup>b</sup>	5.42 <sup>ab</sup>	6.00 <sup>b</sup>	6.60
PP	CS	4.22 <sup>cb</sup>	3.76 <sup>d</sup>	4.03 <sup>e</sup>	5.79 <sup>d</sup>	6.68
PP	ES	3.73 <sup>d</sup>	4.40 <sup>c</sup>	4.84 <sup>cd</sup>	5.45 <sup>e</sup>	6.60
PO	CS	4.30 <sup>b</sup>	4.53 <sup>bc</sup>	5.61 <sup>a</sup>	5.56 <sup>e</sup>	6.78
PO	ES	4.33 <sup>b</sup>	4.49 <sup>c</sup>	5.08 <sup>bc</sup>	5.54 <sup>e</sup>	6.51
PF	CS	4.30 <sup>b</sup>	4.55 <sup>bc</sup>	5.41 <sup>ab</sup>	5.45 <sup>e</sup>	6.74
PF	ES	4.15 <sup>bc</sup>	4.65 <sup>bc</sup>	4.87 <sup>cd</sup>	5.48 <sup>e</sup>	6.95
SE		±0.06	±0.09	±0.07	±0.02	±0.12

<sup>a-†</sup> Least squares means within a column with different superscripts differed significantly ( $P < 0.05$ ).

<sup>†</sup> Coliform count (log Colony Forming Units/g) reported as least squares means along with  $\pm$  standard error.

<sup>‡</sup> Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

<sup>§</sup> Application methods: CS = conventional spray application, ES = electrostatic spray application.

**Table 2. Effects of antimicrobial treatment, application method and day of display against *Escherichia coli* in ground beef during simulated retail display storage at 4 °C.**

Treatment <sup>‡</sup>	Application Method <sup>§</sup>	<i>Escherichia coli</i> count (Log CFU/g) <sup>†</sup>				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	5.22 <sup>a</sup>	5.77 <sup>ab</sup>	6.29 <sup>a</sup>	6.30 <sup>a</sup>	7.33 <sup>a</sup>
PA	CS	3.77 <sup>f</sup>	4.88 <sup>c</sup>	4.96 <sup>f</sup>	6.19 <sup>ab</sup>	7.19 <sup>b</sup>
W	CS	4.22 <sup>d</sup>	5.90 <sup>a</sup>	6.02 <sup>b</sup>	6.31 <sup>a</sup>	7.28 <sup>a</sup>
W	ES	4.43 <sup>c</sup>	5.71 <sup>b</sup>	5.73 <sup>c</sup>	6.28 <sup>ab</sup>	7.13 <sup>c</sup>
PM	CS	4.35 <sup>cd</sup>	3.82 <sup>e</sup>	4.09 <sup>g</sup>	5.49 <sup>d</sup>	7.13 <sup>c</sup>
PM	ES	4.39 <sup>cd</sup>	4.68 <sup>d</sup>	5.44 <sup>d</sup>	6.27 <sup>ab</sup>	6.96 <sup>d</sup>
PP	CS	3.99 <sup>e</sup>	3.84 <sup>e</sup>	4.03 <sup>g</sup>	5.60 <sup>cd</sup>	7.01 <sup>d</sup>
PP	ES	4.81 <sup>b</sup>	4.62 <sup>d</sup>	4.95 <sup>f</sup>	5.77 <sup>cd</sup>	7.21 <sup>b</sup>
PO	CS	4.47 <sup>c</sup>	4.72 <sup>d</sup>	5.95 <sup>b</sup>	5.80 <sup>cd</sup>	6.97 <sup>d</sup>
PO	ES	4.45 <sup>c</sup>	4.67 <sup>d</sup>	5.14 <sup>e</sup>	5.70 <sup>cd</sup>	7.12 <sup>c</sup>
PF	CS	4.32 <sup>cd</sup>	4.62 <sup>d</sup>	5.81 <sup>c</sup>	5.92 <sup>bc</sup>	7.12 <sup>c</sup>
PF	ES	4.29 <sup>cd</sup>	4.77 <sup>cd</sup>	5.06 <sup>e</sup>	5.76 <sup>cd</sup>	7.11 <sup>c</sup>
SE		±0.04	±0.03	±0.02	±0.08	±0.01

<sup>a-g</sup> Least squares means within a column with different superscripts differed significantly ( $P < 0.05$ ).

<sup>†</sup> *Escherichia coli* count (log Colony Forming Units/g) reported as least squares means along with  $\pm$  standard error.

<sup>‡</sup> Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

<sup>§</sup> Application methods: CS = conventional spray application, ES = electrostatic spray application.

**Table 3. Effects of antimicrobial treatment, application method and day of display against total aerobic plate bacteria in ground beef during simulated retail display storage at 4 °C.**

Treatment <sup>‡</sup>	Application Method <sup>§</sup>	Aerobic plate count (Log CFU/g) <sup>†</sup>				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	5.52 <sup>b</sup>	6.27 <sup>a</sup>	6.33 <sup>a</sup>	6.59 <sup>abc</sup>	7.89 <sup>a</sup>
PA	CS	4.57 <sup>f</sup>	4.95 <sup>d</sup>	6.26 <sup>ab</sup>	6.34 <sup>bcd</sup>	7.56 <sup>b</sup>
W	CS	5.04 <sup>d</sup>	6.19 <sup>a</sup>	6.08 <sup>abc</sup>	6.80 <sup>a</sup>	7.52 <sup>bc</sup>
W	ES	5.64 <sup>a</sup>	5.66 <sup>b</sup>	6.21 <sup>ab</sup>	6.68 <sup>ab</sup>	7.24 <sup>d</sup>
PM	CS	5.48 <sup>b</sup>	4.09 <sup>ef</sup>	5.89 <sup>bcde</sup>	5.67 <sup>hg</sup>	7.32 <sup>cd</sup>
PM	ES	5.47 <sup>b</sup>	4.92 <sup>d</sup>	5.68 <sup>de</sup>	6.33 <sup>bcd</sup>	7.36 <sup>bcd</sup>
PP	CS	4.36 <sup>g</sup>	4.00 <sup>f</sup>	4.28 <sup>g</sup>	5.94 <sup>efg</sup>	7.54 <sup>b</sup>
PP	ES	5.11 <sup>d</sup>	4.14 <sup>e</sup>	5.14 <sup>f</sup>	6.12 <sup>def</sup>	7.51 <sup>bc</sup>
PO	CS	4.60 <sup>f</sup>	5.04 <sup>c</sup>	5.98 <sup>abcd</sup>	6.28 <sup>cde</sup>	7.38 <sup>bcd</sup>
PO	ES	4.77 <sup>e</sup>	5.05 <sup>c</sup>	5.59 <sup>e</sup>	5.94 <sup>efg</sup>	7.30 <sup>d</sup>
PF	CS	5.30 <sup>c</sup>	4.11 <sup>e</sup>	5.98 <sup>abcd</sup>	5.86 <sup>fgh</sup>	7.50 <sup>bc</sup>
PF	ES	4.79 <sup>e</sup>	4.06 <sup>ef</sup>	5.74 <sup>cde</sup>	5.57 <sup>h</sup>	7.37 <sup>bcd</sup>
SE		±0.02	±0.02	±0.07	±0.07	±0.04

<sup>a-h</sup> Least squares means within a column with different superscripts differed significantly ( $P < 0.05$ ).

<sup>†</sup> Total aerobic bacterial count (log Colony Forming Units/g) reported as least squares means along with  $\pm$  standard error.

<sup>‡</sup> Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

<sup>§</sup> Application methods: CS = conventional spray application, ES = electrostatic spray application.

**Table 4. Effects of antimicrobial treatment, application method and day of display against *Salmonella* Typhimurium in ground beef during simulated retail display storage at 4 °C.**

Treatment <sup>‡</sup>	Application Method <sup>§</sup>	<i>Salmonella</i> count (Log CFU/g) <sup>†</sup>				
		Day 0	Day 1	Day 2	Day 3	Day 7
CON	-	5.06 <sup>a</sup>	5.58 <sup>a</sup>	6.24 <sup>a</sup>	6.30 <sup>a</sup>	7.54 <sup>a</sup>
PA	CS	3.30 <sup>f</sup>	3.88 <sup>ed</sup>	5.01 <sup>d</sup>	6.19 <sup>ab</sup>	6.63 <sup>d</sup>
W	CS	4.11 <sup>bc</sup>	5.46 <sup>a</sup>	6.27 <sup>a</sup>	6.31 <sup>a</sup>	6.59 <sup>d</sup>
W	ES	4.24 <sup>bc</sup>	4.31 <sup>cd</sup>	5.45 <sup>b</sup>	6.28 <sup>ab</sup>	6.84 <sup>cd</sup>
PM	CS	4.14 <sup>bc</sup>	3.52 <sup>fg</sup>	3.86 <sup>i</sup>	5.49 <sup>d</sup>	6.80 <sup>cd</sup>
PM	ES	3.39 <sup>ef</sup>	3.67 <sup>fg</sup>	4.82 <sup>e</sup>	6.27 <sup>ab</sup>	7.27 <sup>ab</sup>
PP	CS	3.88 <sup>cd</sup>	3.44 <sup>g</sup>	3.72 <sup>j</sup>	5.60 <sup>cd</sup>	6.12 <sup>e</sup>
PP	ES	3.15 <sup>f</sup>	3.56 <sup>fg</sup>	4.17 <sup>g</sup>	5.77 <sup>cd</sup>	7.14 <sup>bc</sup>
PO	CS	3.71 <sup>de</sup>	3.71 <sup>fg</sup>	4.22 <sup>g</sup>	5.80 <sup>cd</sup>	7.02 <sup>bc</sup>
PO	ES	4.27 <sup>b</sup>	4.08 <sup>ed</sup>	4.05 <sup>h</sup>	5.70 <sup>cd</sup>	7.07 <sup>bc</sup>
PF	CS	4.09 <sup>bc</sup>	4.68 <sup>b</sup>	5.26 <sup>c</sup>	5.92 <sup>bc</sup>	7.01 <sup>bc</sup>
PF	ES	3.91 <sup>bcd</sup>	4.62 <sup>bc</sup>	4.64 <sup>f</sup>	5.76 <sup>cd</sup>	7.14 <sup>bc</sup>
<b>SE</b>		±0.08	±0.07	±0.02	±0.07	±0.07

<sup>a-h</sup> Least squares means within a column with different superscripts differed significantly ( $P < 0.05$ ).

<sup>†</sup> Total *Salmonella* species count (log Colony Forming Units/g) reported as least squares means along with  $\pm$  standard error.

<sup>‡</sup> Treatments: CON = untreated inoculated control, PA = 0.02% peroxyacetic acid; W = deionized water, PM = 0.02% peroxyacetic acid followed by 3% malic acid, PP = 0.02% peroxyacetic acid followed by 3% pyruvic acid, PO = 0.02% peroxyacetic acid followed by 3% octanoic acid, PF = 0.02% peroxyacetic acid followed by saturated solution of fumaric acid.

<sup>§</sup> Application methods: CS = conventional spray application, ES = electrostatic spray application.

# Beef quality attributes of precooked ground beef patties formulated with mature bull trimmings

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## Story in Brief

Mature bull necks and A-maturity peeled knuckles were used to test the effect of high pH trim on the cooked color of precooked ground beef patties. Lean (85%) ground beef was formulated with the lean portion consisting of 0%, 25%, 50%, 75%, or 100% bull trim, and the remaining lean portion from USDA Select peeled knuckles, whereas 50% lean trimmings were the “fat” portion. Then, 1/3-lb patties were formed, allowed to bloom 30 min before instrumental color values were collected, refrigerated overnight, and subsequently cooked to an internal temperature of 160 °F in an air-impingement oven, submerged in an ice bath to stop the cooking process, and internal and external instrumental cooked color was measured on 12 random patties/batch. Precooked patties were loosely packaged and frozen until reheating to an internal temperature of 160 °F either in a microwave oven or on gas-fired charbroiler, submerged in an ice water bath, and instrumental cooked color was measured on 12 random patties/batch for each cooking method. Patty pH increased linearly ( $P < 0.001$ ) as the percentage of bull trim increased from 0 to 100%. Raw patties became lighter (greater  $L^*$  values) as the proportion of bull trim decreased (linear,  $P = 0.005$ ). Internal color of the initial cooked patties became darker (lower  $L^*$  values) and more yellow (greater  $b^*$  values) with increasing percentages of bull trim (linear,  $P \leq 0.002$ ). Internal cooked color was lighter ( $P < 0.001$ ) in patties reheated on the charbroiler than in the microwave. Conversely, the internal redness ( $a^*$ ) and yellowness ( $b^*$ ) increased linearly ( $P < 0.001$ ), in reheated patties as the percentage of bull trim increased from 0 to 100%. This could lead to consumer discrimination of precooked ground beef patties, especially those formulated with greater than 50% high pH, mature bull beef trim.

## Introduction

Ground beef is the most common way beef is purchased in the U.S., and is the most commonly consumed form of beef at home as well as away from the home. Precooked ground beef patties are an emerging market because they are convenient for quick, in-home meals, and are a perceived safer product for foodservice outlets. In addition, due to many foodborne outbreaks linked to undercooked ground beef, many of today’s consumers associate a red internal cooked color with questionable wholesomeness. Therefore, the objective of this study was to characterize cooked color of reheated, precooked ground beef patties formulated with various levels of mature bull beef.

## Materials and Methods

Mature bull necks (98% lean), USDA Select peeled knuckles (98% lean), and 50% lean beef trimmings were ground through a 5/8-in plate and mixed to formulate 30-lb batches of 85% lean ground beef. The lean portion was made up of 100%, 75%, 50%, 25%, or 0% mature bull necks (MBT), with the remainder of the lean source being 0%, 25%, 50%, 75%, and 100% peeled knuckles, respectively. Moreover, rosemary extract (antioxidant) was added to each batch at 0.035% of total weight, in addition to 5% tap water. Batches (5 per formulation) were ground through 3/8-in plate and 1/3-lb patties ( $n = 90$ /batch) were formed using a commercial patty forming machine. Six random patties from each batch were selected for raw pH analysis. An additional 12 random patties were allowed to bloom for 30 min before raw color ( $L^*$ ,  $a^*$ , and  $b^*$ ) was measured using a Hunter MiniScan EZ (Hunter Associates Laboratory, Reston, Va.), with illuminant A and a 1-in aperture. Then the 12 segregated patties used for measuring fresh instrumental color were weighed

(to calculate cooking loss), the circumference of each patty was traced onto acetate paper (measure change in patty area), and patty thickness was measured at 4 locations with calipers (monitor change in patty thickness) and, along with other patties from the same batch, cooked to an internal temperature of 160 °F (monitored with a handheld thermometer) in a gas-fired, forced-air, impingement oven set at 400 °F and a belt speed of 10.5 min. After cooking the identified 12 patties were placed into plastic bags and submerged into an ice water bath to stop the cooking process, and subsequently sliced in half (parallel to the surface) exposing the internal surface, and instrumental color ( $L^*$ ,  $a^*$ , and  $b^*$ ) was measured using a Hunter MiniScan EZ, using illuminant A and a 1-in aperture. Three patties from each batch were randomly selected for initial cook pH measurement, whereas all other patties were frozen, loosely bagged, and stored in a freezer.

Twenty-four random patties from each batch were weighed, traced, and patty thickness measured as described previously. These patties were evenly split into 1 of 2 reheating methods, a gas-fired, open-hearth charbroiler (gas grill) set at medium-high heat, to an internal temperature of 160 °F, or an 1,100-W microwave oven for 2 min.

After reheating, patties were placed into plastic bags and submerged into an ice water bath to stop the reheating process, sliced in half, and internal color ( $L^*$ ,  $a^*$ , and  $b^*$ ) was again measured with a Hunter MiniScan EZ (illuminant A and a 1-in aperture). An additional 3 randomly selected patties for each batch, and from both reheating methods were collected to measure reheated pH. Raw, cooked, and reheated patty pH was measured by homogenizing 2 g of patty with 20 mL of distilled/deionized water, and the pH of the homogenate was measured with a temperature-compensating pH meter.

All data were analyzed as a completely randomized design with batch ( $n = 5$  per formulation) as the experimental unit. The analysis

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of variance was carried out using the mixed models procedure of SAS (SAS Institute, Inc., Cary, N.C.), with the proportion of MBT, as well as reheating method, as fixed effects and batch as the lone random effect. Least squares means were separated statistically with the PDIF option of SAS. In addition, linear and quadratic contrasts were used to discern the effects of MBT level in patty formulation on color and dimensions of cooked and reheated patties.

## Results

**Patty Composition.** There was an interactive effect (%MBT inclusion  $\times$  Production Step,  $P < 0.0001$ ) for pH (Fig. 1). Within each production step (raw, initial cook, and reheat) pH was greater ( $P < 0.05$ ) with increasing proportions of MBT. Moreover, pH decreased after initial cook and then again during the reheating step within each MBT treatment. The raw pH findings were expected, as higher levels of MBT would result in a higher patty pH; however, little is known about cooked beef pH, especially when considering mature beef as a lean source.

**Raw Color.** Raw patty lightness ( $L^*$ ) values tended to decrease (linear,  $P = 0.079$ ) with increasing levels of MBT (Table 1). Otherwise, there were no ( $P \geq 0.622$ ) effects of MBT level on raw patty  $a^*$  (redness) and  $b^*$  (yellowness) values.

**Initial Cook Color and Cooking Yields.** Cooked patties became darker (higher  $L^*$  values); and more yellow (higher  $b^*$  values) as the proportions of MBT increased in the patty formulation (linear,  $P \leq 0.002$ ; Fig. 2). Conversely, redness ( $a^*$ ) values of initial cooked patties were not ( $P = 0.50$ ) affected by the amount of MBT in the formulation.

Initial cook losses increased (linear,  $P < 0.0001$ ) as the proportion of MBT increased in the patty (Table 2). Cooked patty thickness changed linearly ( $P < 0.0001$ ) as MBT increased from 0% to 100% in the formulation (Table 2). In fact, 0% MBT patty thickness increased 0.02 in., whereas patty thickness decreased 0.01, 0.04, 0.05, and 0.06 in. for patties formulated with 25%, 50%, 75%, and

100% MBT, respectively (Table 2). In addition, patty area decreased quadratically, ( $P < 0.0001$ ), with the greatest reduction in patty area observed in 50% MBT patties and the least in 0% MBT patties.

**Reheated Color and Cooking Yields.** Reheated patties formulated with 100% and 75% MBT presented greater internal  $a^*$  (linear,  $P < 0.0001$ ) values when compared to 50% to 0% MBT patties (Fig. 3). Moreover, 100% MBT patties were the most yellow (quadratic,  $P < 0.0001$ ) internally of all treatments, whereas 50% patties were less yellow than 25% and 75% MBT patties (Table 3). Also, patties reheated on the charbroiler exhibited greater internal  $L^*$  values ( $P < 0.05$ ), whereas patties formulated with 100% MBT tended to be darker ( $P = 0.07$ ) than 25% MBT patties (Table 3).

When reheated, patties formulated with 100%, 75%, and 0% MBT exhibited lesser cooking losses ( $P < 0.05$ ) than 25% MBT patties (Table 3). Interestingly, patty thickness, when reheated with charbroiler, increased ( $P < 0.05$ ), or "plumped," an average of 0.09-in., whereas patty thickness when reheated with microwave oven decreased ( $P < 0.05$ ) 0.03-in. (Table 4). Regardless of the proportion of MBT, the change in patty area of patties reheated on the charbroiler was less ( $P < 0.05$ ) than ground beef patties reheated in microwave ovens; however, area of 0% MBT patties changed the least when cooked on the charbroiler but had the greatest change in area when cooked in the microwave (Fig. 4). Moreover, 50% and 0% MBT patties when reheated in microwave ovens presented the greatest decrease in area (MBT  $\times$  cookery method,  $P < 0.0001$ ).

## Implications

Although high levels of bull trim have minimal effects on raw ground beef color, results of this study indicated that ground beef patties with the highest proportions of bull trim appeared undercooked even after cooking twice to 160 °F. This could lead to consumer discrimination of precooked ground beef patties, especially those formulated with greater than 50% high-pH, mature-bull beef trim.

**Table 1. Main effects of mature bull beef trimmings (MBT) inclusion on raw ground beef patty color.**

	Mature bull trimmings, %					SEM	Linear <sup>†</sup>
	100	75	50	25	0		
No. of patties	60	60	60	60	60		
Lightness ( $L^*$ ) <sup>‡</sup>	52.04 <sup>c</sup>	52.73 <sup>bc</sup>	54.04 <sup>abc</sup>	55.05 <sup>ab</sup>	55.78 <sup>a</sup>	0.996	0.005
Redness ( $a^*$ ) <sup>‡</sup>	27.25	26.81	25.64	24.66	23.67	2.091	0.175
Yellowness ( $b^*$ ) <sup>‡</sup>	23.31	23.36	23.08	22.87	22.67	0.706	0.436

<sup>a-c</sup> Within a row, least squares means lacking common superscripts differ,  $P < 0.05$ .

<sup>†</sup>  $P$ -value for the linear contrast.

<sup>‡</sup>  $L^*$  = measure of darkness to lightness (larger value indicates a lighter color);  $a^*$  = measure of redness (larger value indicates a more intense red color); and  $b^*$  = measure of yellowness (larger value indicates a more yellow color).

**Table 2. Main effect of mature bull beef trimmings (MBT) inclusion on initial cooking loss and dimension change.**

	Mature bull trimmings, %					SEM	Linear <sup>†</sup>
	100	75	50	25	0		
No. of patties	60	60	60	48	60		
Initial cook loss <sup>‡</sup> , %	38.6 <sup>a</sup>	36.0 <sup>b</sup>	35.9 <sup>b</sup>	34.6 <sup>c</sup>	31.7 <sup>d</sup>	0.003	< 0.001
Initial cooked thickness change <sup>§</sup> , in.	-0.06 <sup>c</sup>	-0.05 <sup>c</sup>	-0.005 <sup>b</sup>	-0.01 <sup>b</sup>	0.02 <sup>a</sup>	0.212	< 0.001
Initial cooked change in patty area <sup>¶</sup> , in. <sup>2</sup>	-4.31 <sup>b</sup>	-4.45 <sup>b</sup>	-5.08 <sup>d</sup>	-4.67 <sup>c</sup>	-3.59 <sup>a</sup>	0.489	< 0.001

<sup>a-c</sup> Within a row, least squares means lacking common superscripts differ,  $P < 0.05$ .

<sup>†</sup>  $P$ -value for the linear contrast.

<sup>‡</sup> Initial cook loss = (Raw patty weight – cooked patty weight/raw patty weight) × 100.

<sup>§</sup> Initial cooked thickness change = raw patty thickness – cooked patty thickness.

<sup>¶</sup> Initial cooked change in patty area = raw planar patty area – cooked planar patty area.

**Table 3. Main effect of mature bull beef trimmings (MBT) inclusion on internal color, cooking loss, and dimension changes of reheated patties.**

	Mature bull trimmings, %					SEM	Linear <sup>†</sup>
	100	75	50	25	0		
No. of patties	60	60	60	48	60		
Internal cooked color							
Lightness ( $L^*$ ) <sup>‡</sup>	61.75 <sup>b</sup>	62.50 <sup>ab</sup>	62.40 <sup>ab</sup>	63.08 <sup>a</sup>	62.32 <sup>ab</sup>	0.327	0.073
Yellowness ( $b^*$ ) <sup>‡</sup>	18.81 <sup>a</sup>	18.45 <sup>b</sup>	18.10 <sup>c</sup>	18.13 <sup>b</sup>	18.22 <sup>bc</sup>	0.095	< 0.001
Dimension changes							
Reheated cooking loss <sup>§</sup> , %	16.4 <sup>b</sup>	15.3 <sup>b</sup>	19.1 <sup>ab</sup>	21.3 <sup>a</sup>	17.0 <sup>b</sup>	0.015	0.099
Reheated thickness change <sup>¶</sup> , in.	0.02	0.03	0.04	0.05	0.03	0.190	0.085

<sup>a-c</sup> Within a row, least squares means lacking common superscripts differ,  $P < 0.05$ .

<sup>†</sup>  $P$ -value for the linear contrast.

<sup>‡</sup>  $L^*$  = measure of darkness to lightness (larger value indicates a lighter color); and  $b^*$  = measure of yellowness (larger value indicates a more yellow color).

<sup>§</sup> Reheat loss = (frozen cooked patty weight – reheated patty weight/frozen cooked patty weight) × 100.

<sup>¶</sup> Reheat thickness change = frozen patty thickness – reheated patty thickness.

**Table 4. Main effect of reheating method on internal cooked color, cooking loss, and dimension changes of precooked ground beef patties.**

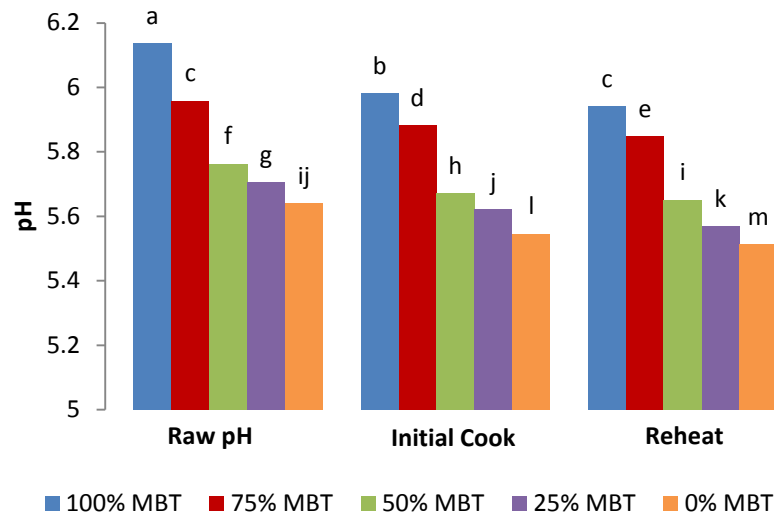
	Reheating method		SEM	$P > F$
	Charbroiler	Microwave oven		
No. of patties	288	288		
Internal cooked color				
Lightness ( $L^*$ ) <sup>†</sup>	62.99	61.82	0.190	< 0.001
Redness ( $a^*$ ) <sup>†</sup>	12.63	12.47	0.234	0.613
Yellowness ( $b^*$ ) <sup>†</sup>	18.31	18.37	0.055	0.410
Dimension changes				
Reheated cooking loss <sup>‡</sup> , %	17.4	18.3	0.008	0.460
Reheated thickness change <sup>§</sup> , in.	0.10	-0.03	0.120	< 0.001

<sup>†</sup>  $L^*$  = measure of darkness to lightness (larger value indicates a lighter color);  $a^*$  = measure of redness (larger value indicates a more intense red color); and  $b^*$  = measure of yellowness (larger value indicates a more yellow color).

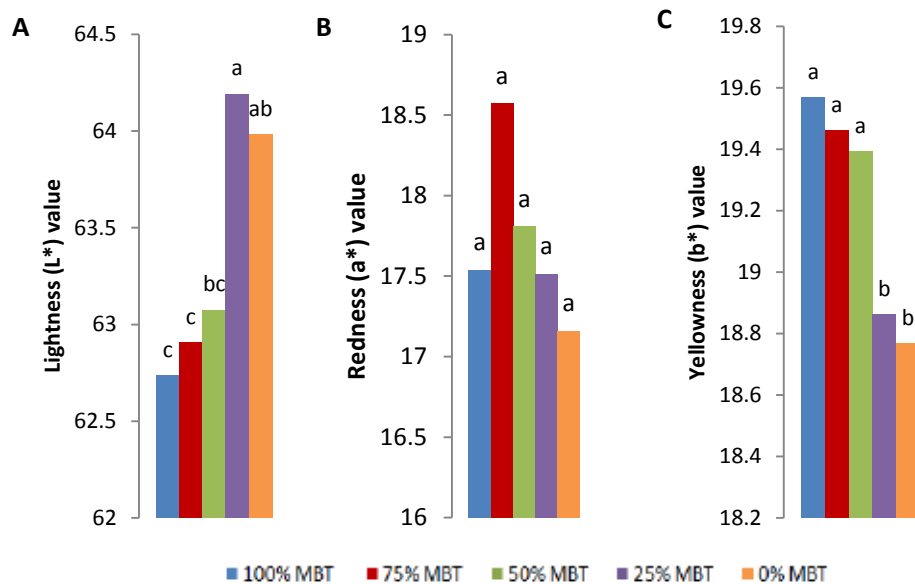
<sup>‡</sup> Reheat loss = (frozen cooked patty weight – reheated patty weight/frozen cooked patty weight) × 100.

<sup>§</sup> Reheat thickness change = frozen patty thickness – reheated patty thickness.

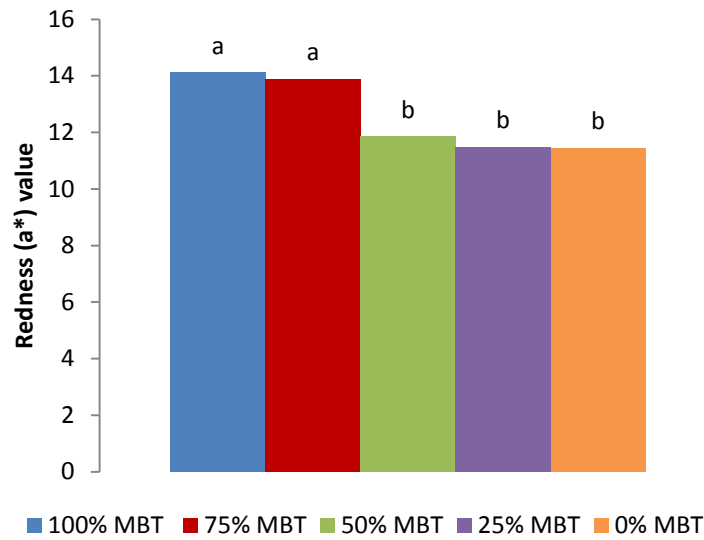




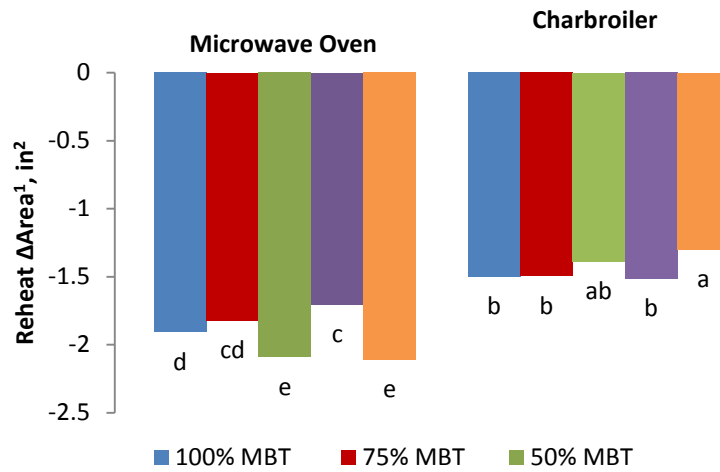
**Fig. 1. Interactive effect of %MBT and production step ( $P < 0.0001$ ) on pH. Bars for 100%, 75%, 50%, 25%, and 0% MBT represent the mean of 6 (Raw), 3 (Initial), and 3 (Reheat) patties per treatment, respectively. Bars lacking common letters (a-c) differ,  $P < 0.05$ ).**



**Fig. 2. Main effects of %MBT inclusion of internal instrumental initial cook ground beef patty color (linear,  $P < 0.0018$ ) on A) lightness ( $L^*$ ) values, B) redness ( $a^*$ ) values, and C) yellowness ( $b^*$ ) values. Bars for 100%, 75%, 50%, 25%, and 0% MBT represent the mean of 60, 60, 60, 48, and 60 patties, respectively. Bars lacking common letters (a-c) differ,  $P < 0.05$ ).**



**Fig. 3. Main effect of %MBT inclusion of internal instrumental reheated ground beef patty color (linear,  $P < 0.0001$ ) on redness ( $a^*$ ) values. Bars for 100%, 75%, 50%, 25%, and 0% MBT represent the mean of 60, 60, 60, 48, and 60 patties, respectively. Bars lacking common letters (a-c) differ,  $P < 0.05$ ).**



**Fig. 4. Interactive effect of %MBT and reheating method ( $P < 0.0001$ ) on  $\Delta$ Area. Bars for 100%, 75%, 50%, 25%, and 0% MBT represent the mean of 60, 60, 60, 48, and 60 patties, respectively. Bars lacking common letters (a-e) differ,  $P < 0.05$ . <sup>1</sup>Reheat  $\Delta$ Area = Frozen planar patty area – reheated planar patty area.**

# Effects of lactic acid enhancement on beef quality attributes of mature bull strip loins

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## Story in Brief

Beef from mature bulls was used to test the effects of lactic acid enhancement solution pH (2.5, 3.0, or 3.5) on fresh and cooked color and tenderness of strip loin steaks. Treatments included a non-enhanced USDA Select control, a non-enhanced bull control, and bull strip loin sections injected with pH 2.5, 3.0, or 3.5 solutions. After enhancement, strip loin sections were vacuum-tumbled, and vacuum-packaged overnight before fabrication into 1-in-thick steaks that were either aerobically packaged and placed into simulated retail display for 5 d, or vacuum-packaged and frozen for Warner-Bratzler shear force and myofibril fragmentation index. Steaks were cooked to 160 °F, evaluated for cooked color within 2 min of slicing, and cores from each steak were used to measure shear force. Steaks enhanced with pH 2.5 and 3.5 solutions tended to have lower ( $P = 0.06$ ) post-enhancement pH values than non-enhanced bull sections, but the pH of enhanced sections was similar to Select. On d 0 of display, Select steaks were redder (higher  $a^*$ ) than non-enhanced bull steaks, but, on d 4 and 5, Select steaks were less red than all mature bull steaks (treatment  $\times$  time,  $P = 0.003$ ). Instrumental cooked color was similar ( $P \geq 0.08$ ) among the treatments; however, Select and 3.5 pH-enhanced bull steaks received greater ( $P < 0.05$ ) visual cooked color scores than non-enhanced bull steaks. Select steaks had greater ( $P < 0.05$ ) myofibril fragmentation index and lower ( $P < 0.05$ ) shear force values than steaks from bull strip loins, regardless of lactic acid enhancement solution pH. Results suggest that lactic acid enhancement of bull steaks improved fresh and cooked color attributes similar to Select, but solution pH failed to produce shear force values comparable to Select.

## Introduction

A combination of drought and high market returns has led to a large influx of mature cattle into the market place. In 2012, bulls represented 1.7% of domestic beef slaughter. Beef production from intact males meets strong resistance from beef packers, resulting from lower USDA quality grades and a belief of lower consumer appeal because of a darker color, coarser texture, and less marbling.

Most bull beef research has involved young maturity bulls and antemortem interventions to improve beef quality; however, little research can be found concerning the effects of maturity on the quality characteristics of fresh and cooked bull steaks. Therefore, the objective of this study was to determine the effects of lactic acid injection on the fresh pH and color stability, as well as cooked color, shear force, cooking loss, and postmortem fragmentation of steaks from mature bulls.

## Materials and Methods

Mature bull (C-, D-, and E-maturity) beef strip loins ( $n = 8$ ) were identified and purchased from a commercial slaughter facility (San Angelo Packing Co., San Angelo, Texas), and vacuum-packaged strip loins were transported back to the University of Arkansas. In addition, A-maturity, USDA Select (SEL) strip loins ( $n = 4$ ) were purchased and served as a non-enhanced, positive control. After aging all strip loins for 12 d at 36 °F, they were sectioned transversely into 2 equal-length sections, and allotted randomly to 1 of 5 treatments: 1) a non-enhanced SEL ( $n = 8$  sections); 2) non-enhanced bull (B0;  $n = 4$  sections); 3) enhanced with a pH 2.5 solution (B25;  $n = 4$  sections); 4) enhanced with a pH 3.0 solution (B30;  $n = 4$  sections); and 5) enhanced with a pH 3.5 solution (B35;  $n = 4$  sections). Treatment enhancement solutions were prepared by titrating lactic acid (88% lactic acid; Purac America, Lincolnshire, Ill.) into buffered (0.25% sodium bicarbonate; Newly Weds Foods, Inc., Chicago, Ill.) tap water. Each strip loin section was weighed,

and sections allotted to the B25, B30, and B35 treatments were injected to 111% of their individual green weight with the assigned enhancement solution via a multi-needle injection, immediately followed by vacuum tumbling for 10 min. Then, loin sections were allowed to drip on racks for 15 min before being vacuum packaged and held overnight at 36 °F. Strip loin sections were subsequently cut into 1-in-thick steaks designated for simulated retail display, myofibril fragmentation index (MFI), cooked color, and Warner-Bratzler shear force (WBSF). Longissimus muscle (LM) pH was measured before and after enhancement with a spear-tip probe and temperature-compensating meter (Testo 205; Testo Ltd., Alton, Hampshire, UK).

For display color, steaks were packaged on foam trays and covered with PVC film, before being placed in simulated retail display (34 °F), and case position was randomly shuffled daily. Measurements of lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) were taken on each day of display. Three scans were taken on each steak using a Hunter Miniscan XE (Hunter Associates Laboratory Inc., Reston, Va.), with a 1-in. aperture and illuminant A.

Steaks for MFI, an indicator of postmortem tenderization, were thawed overnight at 34 °F and then processed according to Culler et al. (1978). Steaks for WBSF were thawed overnight at 34 °F and cooked to 160 °F on countertop electric griddles turning every 2 min, and monitored with a handheld thermometer. Six 0.5-in.-diameter cores were removed from each cooked steak, and shear force was measured using an Instron Universal Testing Machine (Instron Corp., Canton, Mass.) with a 200-lb load cell and Warner-Bratzler shear attachment.

Steaks for cooked color were thawed and cooked as previously described for WBSF. Immediately after cooking, steaks were placed in plastic bags and submerged in an ice bath to stop the cooking process. Each steak was cut transversely, and internal cooked color and degree of doneness were assessed by a 3-person trained panel. Immediately after internal cooked color was visually assessed,  $L^*$ ,  $a^*$ , and  $b^*$  values were measured with a Hunter MiniScan XE with

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a ½-in. aperture and illuminant A. Steaks were also weighed before and after cooking to calculate cooking loss.

Data were analyzed as a completely randomized design, with strip loin section as the experimental unit. Analysis of variance was conducted using the mixed models procedure of SAS (SAS Institute, Inc., Cary, N.C.). Color data were analyzed as repeated measures, with day of display the repeated subject. Least squares means were separated statistically with the PDIF option of SAS.

## Results and Discussion

Mature bull strip loins were expected to have ultimate muscle pH values in excess of 6.0; whereas, beef from young steers and heifers typically have muscle pH values between 5.4 and 5.7. So, the purpose of the lactic acid enhancement was to lower the muscle pH of mature bull strip loins, and, in fact, post-injection pH values tended ( $P = 0.06$ ) to be less in B25 and B35 enhanced strip loins than the non-enhanced B0 strip loins (Table 1).

Steaks from B0, B25, and B30 were darker (lesser  $L^*$  values) and less yellow (lesser  $b^*$  values) than SEL strip steaks (Table 1). Generally, redness ( $a^*$ ) values increased between d 0 and 1 of display before declining on d 2 and 3, and  $a^*$  values of B0, B30, and B35 steaks appeared to level off on d 4 and 5 of simulated retail display. Yet, on d 4 and 5 of display,  $a^*$  values continued to decrease for SEL strip steaks, which were the least red (lowest  $a^*$  values) among all treatments (treatment  $\times$  time,  $P < 0.0029$ ; Fig. 1). These findings indicate that the red, fresh beef color of mature bull beef was extremely stable during the 5 d of simulated retail display.

Regardless of solution pH, MFI values were lower ( $P < 0.05$ ) in mature bull strip loins (Table 2). Furthermore, mature bull strip steaks had greater ( $P < 0.05$ ) WBSF values than SEL strip steaks (Table 2).

Strip steaks injected with solution pH 2.5 and 3.5 had greater ( $P < 0.05$ ) cooking losses than B0 and SEL strip steaks (Table 2).

In addition, there were no ( $P \geq 0.45$ ) differences in cooked redness ( $a^*$ ) and yellowness ( $b^*$ ) values; however, SEL strip steaks tended ( $P = 0.078$ ) to be lighter (greater  $L^*$  values) than steaks from B0, B25, and B30 strip loins. Moreover, B0 strip steaks received lower ( $P < 0.05$ ) cooked color (very red), and degree of doneness (very rare) scores than SEL and B35 strip loins. It should be noted that cooked color and degree of doneness scores were similar ( $P < 0.05$ ) between SEL and B35 strip loins, indicating that the persistent red color associated with high pH beef can be eliminated by lactic acid enhancement (Apple et al., 2011; Sawyer et al., 2009).

## Implications

Results of this study indicate that injecting mature bull beef with lactic acid solution can improve both fresh and cooked beef color. However, cooked beef tenderness did not improve with lactic acid enhancement.

## Literature Cited

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**Table 1. Main effects of lactic acid enhancement on pH and fresh color of mature bull strip loin sections.**

	Treatments <sup>†</sup>				SEM	<i>P</i> > <i>F</i>
	B0	B25	B30	B35		
No. of sections	4	4	4	4	8	
Pre-injection pH	6.09 <sup>b</sup>	6.32 <sup>a</sup>	6.10 <sup>b</sup>	5.70 <sup>bc</sup>	5.62 <sup>c</sup>	0.180
Post-injection pH	6.07 <sup>a</sup>	5.43 <sup>b</sup>	5.80 <sup>ab</sup>	5.55 <sup>b</sup>	5.57 <sup>b</sup>	0.200
Fresh beef color						
Lightness ( $L^*$ ) <sup>‡</sup>	29.41 <sup>c</sup>	34.58 <sup>b</sup>	28.10 <sup>c</sup>	36.90 <sup>ab</sup>	38.68 <sup>a</sup>	0.985
Yellowness ( $b^*$ ) <sup>‡</sup>	15.48 <sup>c</sup>	17.49 <sup>b</sup>	17.07 <sup>b</sup>	18.07 <sup>ab</sup>	19.20 <sup>a</sup>	0.450

<sup>a-c</sup> Within a row, least squares means lacking common superscripts differ,  $P < 0.05$ .

<sup>†</sup> B0 = non-enhanced bull strip loin sections; B25 = bull strip loin sections enhanced with a pH 2.5 solution; B30 = bull strip loin sections enhanced with a pH 3.0 solution; B35 = bull strip loin sections enhanced with a pH 3.5 solution; and SEL = non-enhanced, USDA Select strip loins.

<sup>‡</sup>  $L^*$  = measure of darkness to lightness (larger value indicates a lighter color); and  $b^*$  = measure of yellowness (larger value indicates a more yellow color).

**Table 2. Main effects of lactic acid enhancement on cooked beef attributes and cooked color of mature bull strip steaks.**

	Treatments <sup>†</sup>					SEM	P > F
	B0	B25	B30	B35	SEL		
Shear force, kg	4.33 <sup>a</sup>	4.50 <sup>a</sup>	3.86 <sup>a</sup>	4.99 <sup>a</sup>	2.74 <sup>b</sup>	0.387	0.001
MFI <sup>‡</sup>	177.25 <sup>b</sup>	179.88 <sup>b</sup>	189.00 <sup>b</sup>	183.63 <sup>b</sup>	217.19 <sup>a</sup>	10.544	0.020
Cooking loss, % <sup>§</sup>	15.50 <sup>b</sup>	23.75 <sup>a</sup>	19.50 <sup>ab</sup>	23.25 <sup>a</sup>	17.38 <sup>b</sup>	0.017	0.007
Cooked beef color							
Lightness ( $L^*$ ) <sup>¶</sup>	50.65 <sup>b</sup>	50.69 <sup>b</sup>	50.50 <sup>b</sup>	53.79 <sup>ab</sup>	55.82 <sup>a</sup>	1.840	0.078
Redness ( $a^*$ ) <sup>¶</sup>	25.21	24.42	25.52	24.55	26.32	1.185	0.661
Yellowness ( $b^*$ ) <sup>¶</sup>	20.80	22.10	21.90	22.22	23.13	0.993	0.450
Cooked color score <sup>#</sup>	1.88 <sup>c</sup>	2.42 <sup>bc</sup>	2.12 <sup>bc</sup>	2.67 <sup>ab</sup>	3.10 <sup>a</sup>	0.222	0.012
Degree of doneness <sup>**</sup>	1.92 <sup>c</sup>	2.71 <sup>ab</sup>	2.42 <sup>bc</sup>	2.83 <sup>ab</sup>	3.27 <sup>a</sup>	0.221	0.010

<sup>a-c</sup> Within a row, least squares means lacking common superscripts differ,  $P < 0.05$ .

<sup>†</sup> B0 = non-enhanced bull strip loin sections; B25 = bull strip loin sections enhanced with a pH 2.5 solution; B30 = bull strip loin sections enhanced with a pH 3.0 solution; B35 = bull strip loin sections enhanced with a pH 3.5 solution; and SEL = non-enhanced, USDA Select strip loins.

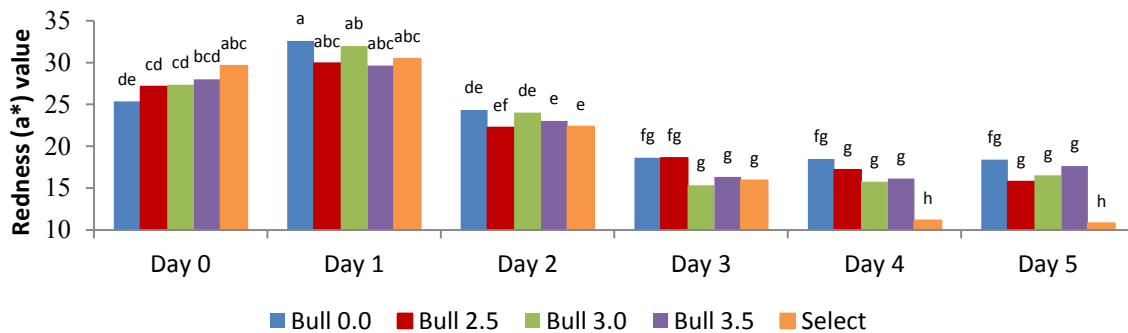
<sup>‡</sup> Myofibrillar fragmentation index (Culler et al., 1978).

<sup>§</sup> Cook loss = (Pre-cook steak weight – post-cook steak weight/pre-cook steak weight) × 100

<sup>¶</sup>  $L^*$  = measure of darkness to lightness (larger value indicates a lighter color);  $a^*$  = measure of redness (larger value indicates a more intense red color); and  $b^*$  = measure of yellowness (larger value indicates a more yellow color).

<sup>#</sup> 1 = very red; 2 = medium red; 3 = pink; 4 = slightly pink; 5 = pinkish gray; 6 = gray brown; and 7 = brown.

<sup>\*\*</sup> 1 = very rare; 2 = rare; 3 = medium rare; 4 = medium; 5 = well done; and 6 = very well.



**Fig. 1. Interactive effects of lactic acid enhancement and duration of simulated retail display ( $P = 0.003$ ) on redness ( $a^*$ ) values. Bars for non-enhanced, mature bull, negative controls (Bull 0.0); mature bull strip loin sections enhanced with solutions of pH 2.5 (Bull 2.5), pH 3.0 (Bull 3.0), and pH 3.5 (Bull 3.5), and non-enhanced, USDA Select, positive controls (Select) represent the mean of 4, 4, 4, 4, and 8 steaks, respectively. Bars lacking common letters (a-h) differ,  $P < 0.05$ .**

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