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Follicular Development of Beef Heifers Exposed to Bulls During an Estrus Synchronization Protocol that Included a 14-Day CIDR, PGF_{2α}, and Timed Artificial Insemination (AI) and GnRH

J.G. Berardinelli*, L.E. Ramos, J.R.C. Wilkinson, J.R. Olsen, T. Gibbs, L. Runnion, and S. A. Tauck¹

Montana State University, Bozeman, MT, 59717

IMPACT STATEMENT

Our results indicate that the continuous presence of mature bulls during an estrus synchronization protocol that included a CIDR for 14 days does not appear to influence ovarian follicular dynamics or the expression of estrus after PG injection in beef heifers. Thus, this may not be the mechanism whereby the presence of bulls increases fertility in the bovine. However, the relationship between the dominate follicle (ovulatory follicle) diameter and body condition score supports the concept that “more fit” females ovulate larger follicles which in turn improves fertility and reproductive efficiency in beef heifers.

SUMMARY

The objective was to evaluate the effect of presence of a bull on ovarian follicular development and its relationship to fertility in beef heifers using an estrus synchronization (ES) protocol that included a progesterone-containing, controlled internal drug release devices (CIDR) for 14 days, PGF_{2α}, and, fixed timed AI (TAI) and GnRH. We found that ovulatory follicle (DF) diameters, at the time of CIDR removal and PGF_{2α} injection (d 0), did not differ between heifers exposed to bulls (BE) and heifers not exposed to bulls (NE). DF diameter of NE and BE heifers were 10.3 ± 0.3 mm (mean ± SE) and 10.9 ± 0.3 mm, respectively. Additionally, there was no difference in number of antral follicles between BE- and NE-treated heifers (1.7 ± 0.1 and 1.5 ± 0.1 follicles, respectively). Diameter of DF did not affect the proportion of heifers that showed estrus or time after PGF_{2α} to exhibition of estrus for heifers in either treatment. Thus, this may not be the mechanism whereby the presence of bulls increases fertility in the bovine. Interestingly, diameters of DF increased (P < 0.05) linearly as body condition score (BCS) increased at a rate of 3.4 mm per unit increase in BCS. This relationship supports the concept that

“more fit” females ovulate larger follicles which in turn might improve fertility in beef heifers.

INTRODUCTION

The occurrence at puberty has a significant effect on reproductive efficiency of beef cattle herds when heifers are bred to calve as 2-yr-olds, particularly in production systems that use restricted breeding seasons (Ferrell, 1982).

Tauck et al., (2007) reported that pregnancy rates increased in primiparous, suckled cows exposed to bulls before and during an ES protocol that included a CIDR for 7 d, PGF_{2α}, and, fixed TAI and GnRH. Based on this and other observations, Berardinelli et al. (2007) suggested that there is the possibility that a pheromone(s) excreted by bulls might be responsible for this enhancement by altering follicular dynamics of ovulatory or dominant follicles (DF).

Previous research indicated that heifers that ovulated follicles >10.7mm and <15.7 mm in diameter at time of AI were more likely to become pregnant than cohorts that ovulated a follicle with a more extreme diameter (Perry et al., 2007). These authors suggested that the use of protocols that control follicular development and increase the likelihood of ovulating optimal sized follicles (10.8 to 15.6 mm) may result in positive benefits on

pregnancy rates in heifers. The biostimulatory effect of the bull might provide this “positive benefit”.

Based on these observations, we postulated that exposing yearling beef heifers to bulls during and ES protocol that included a CIDR for 14 d, PGF_{2α}, and, TAI and GnRH would increase DF diameters at time of CIDR removal and PGF_{2α} injection, and alter the relationship between DF diameters and proportion of heifers showing estrus.

PROCEDURES

Animals and Treatments

Heifers were housed at the Montana State University, Bozeman Area Research and Teaching Facility. Animal care, handling, and protocols used in this experiment were approved by the Montana State University Agricultural Animal Care and Use Committee.

Eight-two, spring-born, Angus X Hereford heifers and two mature epididymectomized Angus X Hereford bulls were used in this experiment. Heifers were maintained in a single pasture and had no contact with bulls or their excretory products from the previous breeding season until the start of the experiment (D -32). Before the start of the experiment, ovarian functional status of each heifer was rated by two ultrasound examinations of each ovary for the presence or absence of a corpus luteum (cycling status). The first and second ultrasonic examinations were conducted 10 and 2 d, respectively, before the start of the experiment. Means for age, BW, and BCS of heifers were 12.8 ± 0.6 months (± SD), 337.3 ± 26.0 kg, and 4.9 ± 0.2, respectively, at the start of the treatment.

Two d before the start of the experiment heifers were stratified by age, BW, BCS, and cycling status. Once stratified, heifers were assigned randomly within strata to one of two treatments: exposed to continuously to bulls EB; n = 41) or not exposed to bulls (NE; n = 41) for 32 d from D -32 to D 0.

Facilities and Bull Exposure

Two lots were used for this experiment, designated north and south by their geographic location. Lots were adjacent to each other and

separated by a wooden, fixed fence and an additional barb-wire fence that separated EB and NE heifers by approximately 15 m. Lots were very similar in east-west configuration, bunk space, aspect, and slope.

The heifer to bull ratio was 10.5:1 throughout the exposure period. Heifers in one pen were prevented from seeing or direct, close contact with bulls by tarpaulins draped over the wooden fence.

Nutrition

Heifers were given 28 lb/hd/d of good quality, chopped mixed-grass alfalfa hay, 2.43 lb/hd/d cracked barley, 1 lb/hd/d supplement that contained 38% protein and 200 mg of Rumensin, water, and a trace mineral-salt supplement throughout the experiment. Heifers were fed one half of the ration in the morning (0800-1000 h) and one half late in the afternoon (1600-1700 h).

Estrus Synchronization Protocol

Each heifer received a progesterone-containing CIDR 32 d before administration of PGF_{2α} (D 0). Fourteen d later (D -18) CIDRs were removed from heifers and each heifer received PGF_{2α} (25 mg/heifer; i.m.). Each heifer was fitted with an estrus detection aid (Estroprotect®; Rockway, Inc., Spring Valley, WI) and observed visually for signs of behavioral estrus. Heifers that showed behavioral estrus and heifers whose estrus-detection aid turned color from silver to red within 60 h after PGF_{2α} were inseminated artificially 12 h later (AI 12). Heifers that did not show estrus and heifers whose estrus-detection aid did not change color within 60 h received GnRH (100 ug/cow; i.m.) and were fixed-time AI (TAI) 72 h after PGF_{2α} (D 3). Heifers that did not exhibit estrus by 60 h after PGF_{2α} were assigned an interval to estrus of 72 h.

Ultrasonography and Follicular Dynamics

Right and left ovaries of each heifer were imaged ultrasonically with at Titan Ultrasound Imaging System (SonoSite, Walla Walla, WA) immediately before PG injection at time of CIDR removal. An image of the ovary containing the largest follicle was capture for morphometric

measurement using Sigma Scan Pro software (SysStat Software, Inc.). An example of the image and measurements to obtain an average diameter are shown in Figure 1.

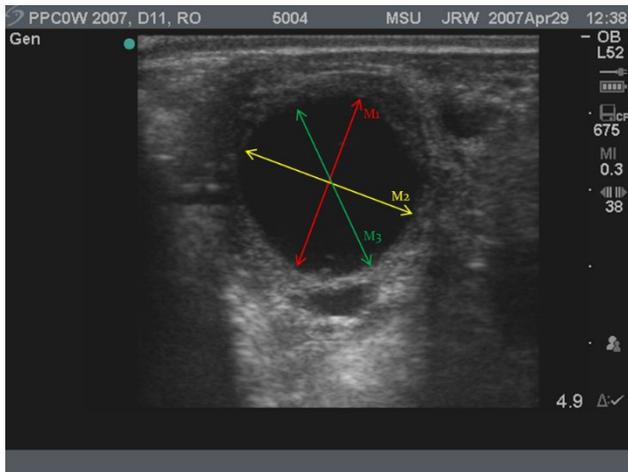


Figure 1. Method for determining measurements of dominant follicle (DF) diameter. Lengths (mm) of intersecting lines were averaged to yield the diameter of the largest follicle on either ovary of each heifer.

Statistical Analyses

Diameters of the largest follicle (DF) and number of follicles were analyzed by separate ANOVA for a completely randomized design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment. Means were separated by Bonferroni's procedure of SAS.

Proportions of heifers that exhibited proportions classified by DF diameters (small, medium, or large) that showed estrus by 60 h after PGF_{2α} separate chi-square analyses using the PROC FREQ procedure of SAS.

Within treatment regressions of DF diameter and body condition score (BCS) were analyzed with PROC REGRESS of SAS.

RESULTS AND DISCUSSION

Diameters of the DF at the time of CIDR removal and PGF_{2α} injection (d 0) did not differ between BE and NE heifers and averaged 10.3 ± 0.3 mm and 10.9 ± 0.3 mm, respectively (Table 1). Furthermore, there was no difference in number of antral follicles > 8.0 mm between BE-

Table 1. Diameters of the dominant follicles (DF) and number of antral follicles >8.0 mm at the time of CIDR removal and PGF_{2α} injection in heifers exposed (BE) and not exposed (NE) to bulls during the ES protocol

Item	Treatment		P-value
	BE	NE	
n	41	41	
DF diam., mm	10.3 ± 0.3	10.9 ± 0.3	0.70
Number antral follicles >8.0mm	1.7 ± 0.1	1.5 ± 0.1	0.50

and NE-treated heifers (1.7 ± 0.1 and 1.5 ± 0.1 , respectively; Table 1).

Diameter of DF did not affect the proportion of heifers that showed estrus or time to estrus of heifers in either treatment (63% and 58% for BE and NE heifers, respectively) or intervals to estrus by 60 h after PGF_{2α} (49.5 ± 6 and 48.8 ± 9 h, respectively).

Interestingly, diameter of DF increased ($P < 0.05$) linearly as BCS increased in BE and NE heifers at an average rate of 3.4 ± 0.7 mm per unit increase in BCS.

In conclusion, the presence of mature bull during an estrus synchronization protocol that included a CIDR for 14 days does not appear to influence ovarian follicular dynamics or the expression of estrus after PGF_{2α} injection in beef heifer. Thus, this may not be the mechanism whereby the presence of bulls increases fertility in the bovine. However, the relationship between DF diameter and BCS supports the concept that "more fit" females ovulate larger follicles which in turn improve fertility.

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*Corresponding author email:
jgb@montana.edu

Identification of Genetic Markers and QTL for Carcass Quality Traits within the American Simmental Association Carcass Merit Program

J. K. Hieber, R. L. Endecott[†], and J. M. Thomson^{*}

Department of Animal and Range Sciences, Montana State University, Bozeman, MT 59717

IMPACT STATEMENT

Beef quality is the driving force behind consumer decisions to consume beef. When quality is poor or inconsistent, genetic improvement can be one strategy to improve meat quality. The American Simmental Association's Carcass Merit Program has allowed us to identify genetic markers that impact carcass traits in Simmental and Simmental-influenced cattle, which could be used to improve the accuracy of breeding value estimations and increase the value of genetic evaluations to Simmental producers

SUMMARY

Genetic improvement of beef product quality benefits both producers and consumers. Beef quality is a leading influence in consumer decisions to consume beef. Insufficient marbling and subsequent lower quality grades, lack of a uniform product, reduced or insufficient tenderness, high carcass weight, or high yield grade can negatively impact beef quality. Improvements can be achieved by making selection decisions based on the results of genetic evaluations in the form of EPDs, real-time ultrasound imaging, and physical evaluation of candidate breeding animals. In an effort to improve their ability to accurately predict the breeding value of potential sires for carcass traits, the American Simmental Association (ASA) launched the Carcass Merit Program (CMP) back in 1997 as a means to collect progeny sire-group carcass information. This program has allowed the ASA to accumulate significant genetic performance information that can reliably be conveyed to producers who utilize Simmental genetics. The objective of this study was to determine genetic markers and QTL in carcass traits from the CMP data collected by the ASA in order to further increase the accuracy of EPD predictions. Data were extracted from the ASA database. Eight chromosomes were found to have genome-wide significance and this

conservative approach yielded putative QTL in regions of these chromosomes.

INTRODUCTION

In 1997, the American Simmental Association (ASA) launched the Carcass Merit Project (CMP), the top sires of the Simmental and Simbrah breeds were randomly mated to commercial females with the plan to collect difficult-to-get progeny sire-group carcass information and sometimes tenderness data. Additionally, information about carcass traits in this program contributed well over 1,000 birth, weaning, and yearling weights and calving ease scores each year, and to date nearly 4,000 shear force records have been collected.

These data have allowed the ASA to amass substantial information necessary for benchmarking, so that performance and value expectations can be conveyed to current and potential customers of ASA's members.

In the last 25 yr, a shift has occurred in the U.S. beef industry from a commodity-based market to one that is based on quality or value added. This has been facilitated by the ability of cattlemen to identify and select animals with superior carcass merit. In an effort to retain market share and increase overall consumer acceptance, many producers have placed at least some importance on selection for marbling development or carcass quality. This means that

a large number of our current cattle herds have females that possess merit in the area of marbling or carcass quality. As a result, there is much interest in how carcass-based selection impacts the maternal performance of these animals. In addition, there is a desire to continue to improve genetic merit for carcass and product consistency. Genomic or marker-based selection is a tool that can be used to improve these traits. With that, our hypothesis is that we can identify genetic markers and QTL for carcass traits from the 30 yrs of CMP data collected by the ASA.

PROCEDURES

Data were generated by the ASA CMP. All records containing calf birth weights, calving ease scores, weaning weights, back fat, ribeye area, marbling, HCW, KPH were extracted from the ASA database. Sire EPD, performance, and genotype data were also extracted.

Progeny data were organized into sire families for all traits, and progeny performance phenotypes were constructed. Sires that had either SNP50K or imputed 50K data were used in the overall analyses. Quality control of samples, markers, and SNP data was done through a series of filters (Golden Helix, 2017).

Principal component analysis (PCA) was used to determine relatedness between individuals. The relatedness was used as a covariate in the association analyses.

A single-locus, mixed linear model (EMMAX; Kang et al., 2010) in SVS software (Golden Helix, version 8.7.2-2017-08-11) was used to analyze the data. Manhattan plots were created to visualize the association analyses. On the Manhattan plots, the genome-wide significance level for the Benjamini-Hochberg correlation with $-\log_{10}(p\text{-value})$ is 5×10^{-8} (Ehret, 2010) and markers that were above the level of significance were considered to be associated with the trait in question. Regions with clusters of significantly associated markers were then labeled as putative QTL and used to identify potential positional candidate genes within each.

RESULTS AND DISCUSSION

The data contained samples from 3,849 individuals. We were able to group individuals by sire, in which 2,745 individuals had known sires, producing 395 sire families. Sire families ranged in size from 1 progeny up to 150 progeny; however, not all of the progeny had reported information for their carcass traits. For those who had reported carcass trait information, progeny average performance phenotypes were constructed.

To identify potential candidate genes and pathways related to each carcass trait, Manhattan plots were created for each trait and were used to identify regions of the genome that associated with the traits. In the Manhattan plots, each new color represents a new chromosome, beginning with chromosome 1 on the left and continuing in ascending order to chromosome 29, and each dot represents a marker.

We found 8 out of 261 total chromosomes to have genome-wide association significant markers. For HCW, chromosomes 6, 7, 14, and 20 had 5, 1, 2, and 1 significant markers, respectively (Figure 1). For KPH, chromosomes 11 and 16 each had one significant marker (Figure 2). Chromosome 20 for average HCW, chromosome 16 for average carcass marbling, and chromosome 17 for average carcass fat each had one significant marker.

In order to identify candidate genes, we used a 100,000 bp window around regions or markers that were significantly associated with the trait of

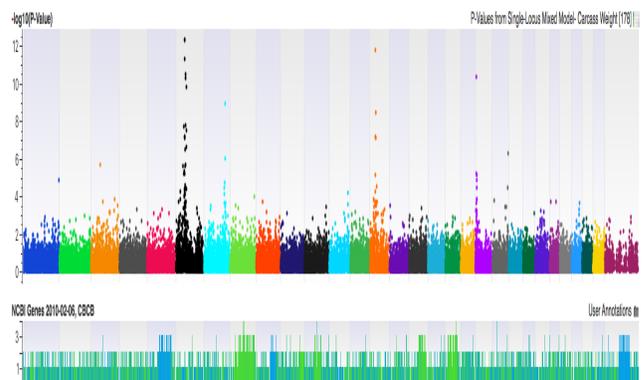


Figure 1. Manhattan plot for hot carcass weight (HCW). Markers above $-\log_{10}(p\text{-value})$ of 5×10^{-8} are genome-wide association significant markers. Vertical clusters of markers are of interest as they indicate putative QTL in those regions.

interest. Regions harboring significant associations were cross-referenced with the University of California-Santa Cruz Genome Browser to determine if known bovine genes were within the regions. Not all significant genomic regions had previously reported positional candidate genes indicating that there are carcass traits that are being impacted by genes not yet identified.

This study demonstrates the value of the ASA CMP for identifying and characterizing genomic variants that impact carcass traits in Simmental and Simmental-influenced cattle, and, can be used to improve the accuracy of breeding value estimations and increase the value of genomic data to Simmental producers. Further research is needed before these results are immediately applicable to the ASA and producers. Additional research needed includes determining the amount of variation in the traits of interest the markers and QTL explain.

Lastly, genetic and phenotypic correlations to other economically important traits, specifically

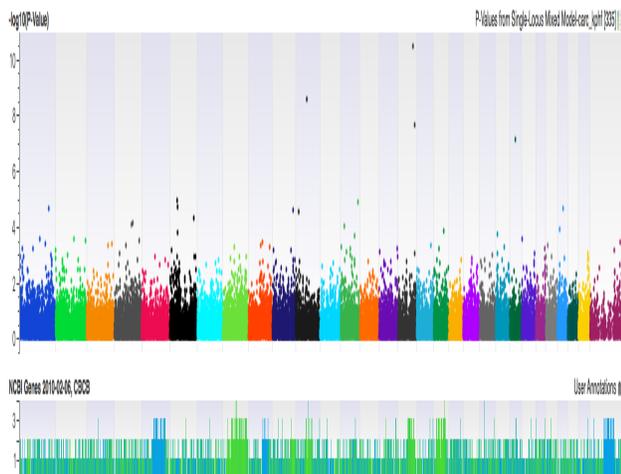


Figure 2. Manhattan plot for kidney, pelvic, heart fat (KPH). Markers above $-\log_{10}(p\text{-value})$ of 5×10^{-8} are genome-wide association significant markers. Vertical clusters of markers are of interest as they indicate putative QTL in those regions.

maternal traits, will be calculated. This will allow for the ASA to advise breeding recommendations to maximize producer profitability by determining the best balance of selective pressure to continue to improve carcass traits while minimizing the negative impacts on other traits.

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†Present address: American Simmental Association, Bozeman, MT 59715

*Corresponding author email: jennifer.thomson@montana.edu

Inoculation and Soil Fertility Management to Optimize Pulse Crop Yield and Protein

C. Jones* and K. Olson-Rutz

Department of Land Resources and Environmental Sciences, Montana State University

IMPACT STATEMENT

Proper inoculation and soil fertility management to encourage nitrogen fixation may be a worthwhile investment for optimal economic pulse crop production.

SUMMARY

Pulse crops require nitrogen from either the soil or nitrogen (N) fixation by rhizobia in root nodules. There are agronomic decisions that promote healthy nodulation and N fixation. We summarize regional research on factors that affect N fixation and pulse seed yield and protein. This report is a synopsis of our paper published in *Crops & Soils Magazine*, Volume 51, Issue 4 (<https://dl.sciencesocieties.org/publications/cns/tocs/51>).

INTRODUCTION

Pulse crops acquire N either from the soil or through N fixation by rhizobia bacteria in nodules on their roots. Given a choice, a plant will generally extract available N from the soil, rather than go through the carbon-expensive relationship with rhizobia. Most soils contain some N-fixing rhizobia; however, an inoculant with rhizobia specific to the pulse crop provides the most effective N fixation.

NODULATION & N FIXATION

Native rhizobia populations are lower and nodule activity is severely limited in dry or waterlogged soil, at extreme soil temperatures, and in acidic and saline soils (Denton et al., 2013), although inoculant strains and their host plants differ in pH and salinity tolerance (Bordeleau and Prevost, 1994). Rhizobia persistence in the soil is reduced under adverse soil conditions, especially when host plants are not present. Nodule activity decreases as plants mature past flowering.

There are several practices which can improve nodulation and N fixation. Liquid and seed-applied peat inoculants need to be kept cool and dark before application. Contact with fertilizer and some fungicides may also be detrimental to inoculants (Kutcher et al., 2002). The pulse/rhizobia system needs adequate phosphorus (P), potassium (K), sulfur (S) and micronutrients for optimal N fixation. Too much soil nitrate inhibits nodulation and N fixation, while no-till enhances N fixation by retaining soil moisture.

INOCULATION

Peat-powder or liquid inoculant applied to the seed is less expensive, but more challenging to properly care for before and during application than soil-applied peat granular inoculant. When compared to seed-applied powder, granular inoculant produced higher protein in Montana especially under drought conditions (Bestwick et al., 2018), higher yields in fields with no pulse history (Gan et al., 2005, SK), higher protein and yield with little or no N-fertilizer (Clayton et al., 2004a), more active nodules at soil pH 4.4, and greater N fixation and plant biomass at soil pH 5.4 (Rice et al., 2000).

Granular soil-applied inoculant promotes rhizobia populations that are evenly distributed on tap and lateral roots, whereas seed-applied results in nodules clustered near the root crown (Figure 1; Rice et al., 2000). Tap and lateral root nodules provide a benefit where surface soils may dry out, or in low pH soils with low populations of indigenous rhizobia. Some



Figure 1. Nodule formation along the length of the tap and lateral roots with granular inoculant (left) and in clusters near the soil surface with seed-applied inoculant (right; photos by P. Miller).

farmers use two forms of inoculant to decrease odds of inoculant failure.

Inoculant is most effective in fields without recent pulse history. An Alberta study found the likelihood of a significant increase in seed yield from granular inoculant was independent of spring soil nitrate-N, yet the magnitude of the response was higher at low soil nitrate-N (18 lb N/acre; McKenzie et al., 2001a). The same study found inoculant is more likely to increase protein under low spring soil nitrate levels (less than 18 lb N/acre) and very unlikely to increase protein under high spring nitrate (greater than 36 lb N/acre).

FERTILIZER N

Nodulation is carbon expensive, meaning it requires healthy plants. Since little N is contributed by nodules until the third node, N for initial growth must come from top foot of soil. Nitrogen hunger can lead to plants getting ‘stuck’; they can’t grow to feed nodules, and nodules aren’t actively providing N for growth. Rhizobia-fed plants in low N soil take two to three weeks longer to get going than pulse plants with adequate soil or fertilizer N (P. Miller, pers. comm.). Finally, fertilizer N is insurance against nodule loss to pea leaf weevil, whose larvae feast on nodules.

There is a balance between enough soil nitrate for plant growth and too much N, which can inhibit nodulation, produce excess early vegetation, and reduce yield. Based on Montana and Alberta studies (McConnell et al., 2002; McKenzie et al., 2001a) it is reasonable to aim

for 10-15 lb total available N/acre (soil + fertilizer) in the top foot in spring, keeping in mind that seed row N may hurt germination. The close proximity of seed row fertilizer N has more negative impact on nodulation than high residual soil nitrate level (Clayton et al., 2004b). Starter N appears to be more important with seed-applied peat-powder inoculant than peat granular inoculant (Clayton et al., 2004a). However, there is no guarantee that starter N will increase either yield or protein.

RESCUE N

If nodulation failure is suspected, rescue N can ‘salvage’ yields if applied by six weeks after seeding (pea, 9-12 node, 7-10 leaf; chickpea, 10-13 node, 8-11 leaf; McConnell et al., 2002, MT). If applied later there is higher risk of too much vegetative growth, poor pod set, and delayed maturity. Rescue N requires rain/irrigation to move N into the soil where it can be taken up by plants. Although starter or rescue N may appear to boost early crop growth, yield gain may not offset N cost.

OTHER NUTRIENTS

Optimizing availability of all nutrients should increase N fixation and yield.

Sulfur is important for N uptake and protein production. Sulfate S can be applied at planting as a side band liquid or granular, saving the seed row for P. Alternatively, bank elemental S a rotation or two prior to the pea rotation (except in acidic soils since elemental S decreases pH). Phosphorus and potassium are important for N fixation and should be supplied based on soil tests and regional guidelines. In general, P response is more likely when Olsen P is less than 9 ppm (Chen et al., 2006; McKenzie et al., 2001b; Karamanos et al., 2003). When Olsen P is greater than 13 ppm, low maintenance rates can be added. If soil tests suggest higher P rates, side band the additional amount at seeding, or build up P with the prior crop. Potassium is generally not limiting in soils of the northern Great Plains, although it should be monitored in rotations with high removal of stems and leaves and in coarse soils. Some K should be added if the soil test K is less than 250 ppm.

ECONOMICS

In low N soils, yield and protein may increase with starter N or inoculant, but until protein is more commonly rewarded with higher prices, the reward may not offset the cost. Yet, the risk of crop failure due to low N or failed nodulation are reasons it may not be worth skimping on inoculant or low rates of starter N. For example, given soil acidification in the soil surface is a growing problem in traditionally neutral to alkaline soils (<http://landresources.montana.edu/soilfertility/acidif.html>), a surface soil pH test and providing granular inoculant if the soil has pH < 6 may pay off.

Factors to consider when selecting inoculant and starter fertilizer are: spring soil nitrate levels, field history with pulses and inoculation, whether water is the likely yield limiting factor, soil conditions that limit nodulation and the indigenous rhizobia population, and whether a premium will be paid for protein.

RESOURCES

IPNI/SDSU Seed Damage Calculator

<http://seed-damage-calculator.herokuapp.com/>

Montana Cool Season Pulse Production Guide

<http://landresources.montana.edu/soilfertility/documents/PDF/pub/MTCool-SeasPulseProd%20EB0210.pdf>

Montana State University Soil Fertility

Extension website for presentations on pulse inoculation and soil nutrient management

<http://landresources.montana.edu/soilfertility/presentations.html>

Pea Protein Formation and Management

Options. 2018. M. Bestwick, P. Miller, C. Jones, and K. Olson-Rutz.

<http://landresources.montana.edu/soilfertility/documents/PDF/reports/Bestwick2018PeaProTFormation.pdf>

SK Pulse Growers' Nodulation and N-Fixation Field Assessment Guide

http://proof.saskpulse.com/files/general/150521_Nodulation_and_Nitrogen_Fixation_Field_Assessment_Guide.pdf

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*Corresponding author email:
clainj@montana.edu

Evaluating the Effect of South African Meat Merino Breeding on Pre- and Post-Weaning Growth, Feedlot Performance, Carcass Traits, and Wool Characteristics

R. M. Knuth¹, W. C. Stewart², J. A. Boles¹, C. M. Page², A. F. Williams¹, and T. W. Murphy^{1*}

¹Department of Animal and Range Sciences, Montana State University, Bozeman 59717 and

²Department of Animal Science, University of Wyoming, Laramie 82071

IMPACT STATEMENT

The South African Meat Merino is a relatively new addition to the U.S. sheep industry and has not been extensively compared to breeds commonly used in Western sheep production. Results from the present study indicate that weaning weight and carcass characteristics were similar between purebred Rambouillet and South African Meat Merino x Rambouillet lambs. However, Rambouillet sired ewes were heavier as yearlings and had lower MFD than South African Meat Merino sired ewes. These results should be interpreted judiciously as a preliminary component of a multi-year study. Future research evaluating lifetime lamb and wool production of South African Meat Merino cross ewes is ongoing with this project.

SUMMARY

The South African Meat Merino (SAMM) is a dual-purpose breed whose selection has focused on lamb and wool production. However, little is known about its ability to enhance these economically important traits in Western sheep production. The objectives of this study were to compare pre- and post-weaning growth, feedlot performance, carcass traits, and fleece characteristics of sheep reared by Rambouillet dams and sired by either Rambouillet, Suffolk, or SAMM rams. Rambouillet sired lambs were 0.4 lbs lighter ($P = 0.04$) at birth than SAMM sired lambs in 2016. In 2017, Suffolk sired lambs were 0.7 and 1.8 lbs heavier ($P \leq 0.01$) at birth than SAMM and Rambouillet sired lambs, respectively, which were not different ($P = 0.12$). No differences ($P \geq 0.38$) in 120 d body weight were observed among sire breeds in either year. The same sire breeds were compared in an 89 d wether feeding trial, and no differences ($P \geq 0.25$) were observed in body weight at the end of the trial or average daily gain and feed conversion ratio throughout the trial. However, Suffolk sired wethers had a higher dressing percentage and heavier leg and loin weights ($P \leq 0.01$) than Rambouillet and SAMM sired wethers, which were not different ($P \geq 0.12$). A

portion of Rambouillet and SAMM sired ewe lambs were kept as replacements to the breeding flock. Rambouillet sired ewes were 9 lbs heavier ($P < 0.01$) as yearlings than SAMM sired ewes. Ewes were shorn at 1 and 2 yr of age and sire breed had no effect ($P \geq 0.40$) on greasy fleece weight or CV of fiber diameter. However, mid-side wool samples of SAMM sired ewes were 2.1 μm coarser in mean fiber diameter ($P = 0.04$) than Rambouillet sired ewes.

INTRODUCTION

Between 2010 and 2015, wool sales represented 6.5 to 13% and lamb sales 76 to 83% of the annual returns to a typical Western rangeland sheep operation (LMIC, 2016). An economically sound breeding program should focus on improving lamb production traits while maintaining wool quality. Mating Western white-faced ewes (e.g., Rambouillet, Targhee) to terminal sire breeds (e.g., Suffolk, Hampshire) has improved lamb pre- and post-weaning growth (Leeds et al., 2012; Notter et al., 2012) and carcass characteristics (Mousel et al., 2012; 2013). However, fleece quality is significantly reduced in these crossbred lambs (Scales et al., 2000) and resulting ewe lambs are not typically kept as replacements. A white-faced, dual-

purpose sire breed that can improve lamb traits without large sacrifices in wool quality would be beneficial to the Western sheep industry.

Selection in the South African Meat Merino (SAMM) has emphasized lamb and wool production (Neser et al., 2000; Cloete et al., 2004b). Cloete and Durand (2000) reported that SAMM x Merino lambs were heavier at birth and weaning but had lighter clean fleece weight, shorter staple length, and higher mean fiber diameter as yearlings than purebred Merinos. The SAMM is a relatively new addition to the U.S. with few purebred flocks and research reports (Meyerhoff et al., 2017). Therefore, the objectives of this study were to compare pre- and post-weaning growth, feedlot performance, carcass traits, and fleece characteristics of sheep reared by Rambouillet dams and sired by either Rambouillet, Suffolk, or SAMM rams.

PROCEDURES

The Montana State University (MSU) Agricultural Animal Care and Use Committee approved all husbandry practices and experimental procedures used in this study. Commercial Rambouillet ewes were exposed to either Rambouillet (n = 5), SAMM (n = 5), or Suffolk rams (n = 2) in November 2015 and 2016 and lambs were born in April of the following years. In both production years, ewes and lambs were managed on native rangeland until weaning.

Approximately 3 mo after weaning in 2016, Rambouillet (n = 10), Suffolk (n = 10), and SAMM (n = 10) sired wethers with a similar body weight (BW) across genotype were placed in a single drylot pen (129 m x 21 m) equipped with four GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada). Lambs were adapted to the GrowSafe bunks and trial diet (15.1% crude protein, 61.6% total digestible nutrients) for 10 d and fed *ad libitum* thereafter. Fasted BW was collected on consecutive days at the start and end of the 89 d feeding trial. Wethers were harvested following the feeding trial.

Rambouillet and SAMM sired replacement ewe lambs were selected at weaning in 2016 and 2017 and managed with the adult ewe flock thereafter. Body weight was collected on 2016 born Rambouillet and SAMM sired ewes at approximately 12 mo of age. Greasy fleece weight (GFW) and mid-side wool samples were collected at shearing (February 2018) on 2016 and 2017 born Rambouillet (n = 45) and SAMM sired (n = 37) replacement ewes. Fiber metrology traits of side samples were analyzed at the MSU Wool Laboratory on an Optical-based Fiber Diameter Analyser 2000.

Statistical Analyses

Lamb BW at birth (n = 405) and 120 d (n = 305) was analyzed within year procedure with fixed effects of sire breed, sex, dam age (2, 3, or 4+ yr), and birth or rear type (single or multiple) and the random effects of dam and sire. In the

Table 1. Least-squares means (\pm SE) for the main effects of birth type (BT) or rear type (RT), sex, age of dam, and sire breed on lamb body weight (BW) at birth and 120 d in the 2016 and 2017 production years.

Effect	Level	2016		2017	
		Birth BW, lbs	120 d BW, lbs	Birth BW, lbs	120 d BW, lbs
BT, RT ¹	1	11.9 \pm 0.22 ^a	67.7 \pm 1.54 ^a	12.8 \pm 0.27 ^a	78.0 \pm 1.61 ^a
	2+	9.7 \pm 0.18 ^b	52.7 \pm 1.79 ^b	10.4 \pm 0.24 ^b	59.3 \pm 1.68 ^b
Sex	Ewe	10.4 \pm 0.18 ^b	58.4 \pm 1.70	11.0 \pm 0.22 ^b	67.2 \pm 1.52
	Wether	11.0 \pm 0.18 ^a	61.7 \pm 1.63	12.1 \pm 0.24 ^a	70.1 \pm 1.65
Age of dam, yr	2	11.0 \pm 0.40	60.2 \pm 3.79	11.0 \pm 0.40	66.6 \pm 2.36
	3	10.4 \pm 0.18	59.3 \pm 1.46	12.3 \pm 0.44	67.2 \pm 5.97
	4+	10.6 \pm 0.15	61.1 \pm 1.08	11.2 \pm 0.20	67.2 \pm 1.32
Sire breed ²	Rambouillet	10.6 \pm 0.15 ^b	60.4 \pm 1.54	10.8 \pm 0.26 ^b	68.3 \pm 1.96
	SAMM	11.0 \pm 0.15 ^a	59.7 \pm 1.79	11.5 \pm 0.33 ^b	67.0 \pm 2.05
	Suffolk	-	-	12.6 \pm 0.33 ^a	70.8 \pm 2.49

¹BT, RT= observed birth type for birth BW or rear type at for 120 d BW.

²Lambs sired by either Rambouillet, South African Meat Merino (SAMM), or Suffolk rams and raised by Rambouillet ewes.

^{a,b}Means within an effect and column are different ($P < 0.05$).

2015 breeding season, Rambouillet ewes were exposed to Suffolk rams in multiple sire groups, therefore, sire identification was not available in 2016 born Suffolk cross lambs and they were not included in the 2016 analysis.

Feedlot lamb BW at the start and end of the trial, average daily gain (ADG), feed conversion ratio (FCR), and carcass characteristics were separately analyzed with the effect of sire breed. Carcass characteristics included: dressing percentage (DP), loin, leg, and rack weights, back fat depth (BF), and loin eye area (LEA). Yearling BW and lamb and yearling GFW, mean fiber diameter (MFD), and CV of fiber diameter (CV-FD) were analyzed separately with the fixed effect of sire breed and the random effect of sire.

RESULTS AND DISCUSSION

Pre-weaning Performance

Least-squares means for the main effects of birth or rear type, sex, age of dam, and sire breed on lamb BW at birth and 120 d in 2016 and 2017 are displayed in Table 1. Not surprisingly, single born/reared lambs were heavier at birth and 120 d ($P < 0.001$) than multiple born/reared lambs in both years. Males were heavier ($P < 0.001$) at birth than females in both years but sex had no effect ($P \geq 0.08$) on 120 d BW in either year. Age of dam did not affect ($P \geq 0.12$) lamb BW at birth or 120 d in either year.

In 2016, SAMP sired lambs were 0.4 lbs heavier ($P = 0.04$) at birth than Rambouillet sired lambs. Suffolk sired lambs born in 2017 were 1.8 and 0.7 kg heavier at birth ($P \leq 0.01$) than Rambouillet and SAMP sired lambs, respectively, which were not different ($P = 0.12$). However, sire breed had no effect ($P \geq 0.38$) on 120 d BW in either year. Cloete and Durand (2000) reported that lambs sired by SAMP rams and reared by Merino ewes were 0.68 and 9.04 lbs heavier at birth and 140 d, respectively, than purebred Merino lambs.

Feedlot Performance and Carcass Characteristics

Least-squares means for the main effect of sire breed on feedlot performance and carcass characteristics are presented in Table 2. Sire breed had no effect on BW at the start or end of the trial, ADG, or FCR ($P \geq 0.25$). Meyerhoff et al. (2017) reported similar results where no difference in BW gain or feed efficiency was observed between purebred and crossbred Rambouillet rams of varying (25-50%) SAMP breeding.

Suffolk sired lambs had the greatest DP (53.7%; $P \leq 0.001$), but DP of Rambouillet (50.0%) and SAMP sired (51.2%) lambs was not different ($P = 0.12$). Suffolk sired lambs also had the heaviest ($P \leq 0.01$) loin (6.86 lbs) and leg weights (12.2 lbs), but these were not different ($P \geq 0.52$) between Rambouillet (6.17 and 10.8 lbs,

Table 2. Least-squares means (\pm SE) for the main effect of sire breed on feedlot performance and carcass characteristics.

Trait ²	Sire Breed ¹		
	Rambouillet	SAMP	Suffolk
Start BW, lbs	86.0 \pm 1.59	85.3 \pm 1.59	87.5 \pm 1.59
End BW, lbs	134.0 \pm 2.34	132.9 \pm 2.34	138.2 \pm 2.34
ADG, lbs d ⁻¹	0.56 \pm 0.02	0.53 \pm 0.02	0.57 \pm 0.02
FCR	10.6 \pm 0.37	10.7 \pm 0.37	10.3 \pm 0.37
DP, %	50.0 \pm 0.51 ^b	51.2 \pm 0.48 ^b	53.7 \pm 0.48 ^a
Leg, lbs	10.8 \pm 0.24 ^b	11.0 \pm 0.24 ^b	12.2 \pm 0.24 ^a
Loin, lbs	6.17 \pm 0.18 ^b	6.13 \pm 0.18 ^b	6.86 \pm 0.18 ^a
Rack, lbs	5.78 \pm 0.13	5.60 \pm 0.13	5.91 \pm 0.13
LEA, in ²	2.36 \pm 0.06	2.43 \pm 0.06	2.54 \pm 0.06

¹Lambs sired by either Rambouillet, South African Meat Merino (SAMP), or Suffolk rams and raised by Rambouillet ewes.

²Start BW = BW at the start of the trial; End BW = BW at the end of the trial; ADG = average daily gain; FCR = feed conversion ratio; DP = dressing percentage; Leg, Loin, and Rack = weight of leg, loin, and rack, respectively; LEA = loin eye area.

^{a,b}Sire breed means within a trait are different ($P < 0.05$).

respectively) and SAMM sired lambs (6.13 and 11.0 lbs, respectively). No difference ($P = 0.28$) in rack weight was observed among sire breeds. Cloete et al. (2008) compared carcass characteristics of terminally sired lambs reared by either purebred SAMM or SAMM x Merino dams and found that while shoulder weight increased with proportion of SAMM breeding, hindquarter and loin weight did not.

Wool Characteristics and Yearling BW

Least-squares means for the main effects of age of ewe and sire breed on wool characteristics are displayed in Table 3. Two-yr-old ewes had heavier GFW, coarser MFD, and lower CV-FD ($P \leq 0.005$) than 1-yr-old ewes. Mid-side wool samples of SAMM sired ewes were 2.1 μm coarser ($P = 0.04$) than Rambouillet sired ewes, but sire breed did not influence GFW or CV-FD ($P \geq 0.40$). Cloete and Durand (2000) reported that SAMM x Merino ewes had 1.79 lbs lighter yearling GFW and 1.5 μm coarser MFD than purebred Merino ewes but did not differ in CV-FD. In the present study, Rambouillet sired ewes were heavier (100.5 lbs; $P < 0.001$) as yearlings than SAMM sired ewes (91.5 lbs). In contrast, Cloete and Durand (2000) reported that SAMM x Merino yearling ewes were 24.3 lbs heavier than purebred Merino yearling ewes.

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Table 3. Least-squares means (\pm SE) for the main effects of sire breed and ewe age on yearling greasy fleece weight (GFW), mean fiber diameter (MFD), and CV of fiber diameter (CF-FD).

	Level	Trait		
		GFW, lbs	MFD, μm	CV-FD, %
Sire breed ¹	Rambouillet	4.41 \pm 0.13	19.9 \pm 0.38 ^b	17.6 \pm 0.44
	SAMM	4.41 \pm 0.13	22.0 \pm 0.40 ^a	17.0 \pm 0.46
Age of ewe, yr	1	3.09 \pm 0.13 ^b	19.7 \pm 0.32 ^b	17.9 \pm 0.38 ^a
	2	5.95 \pm 0.13 ^a	22.2 \pm 0.40 ^a	16.6 \pm 0.40 ^b

¹Ewes sired by either Rambouillet or South African Meat Merino (SAMM) and raised by Rambouillet ewes.

^{a,b}Means within an effect and column are different ($P < 0.05$).

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*Corresponding author email:
thomas.murphy12@montana.edu

Forage Nitrate Analysis: What Method to Use?

E. Meccage^{1*}, Z. Miller², and D. Peterson¹

¹Department of Animal and Range Sciences, Montana State University, Bozeman, MT 59717 and

²Western Agricultural Research Center, Montana State University, Corvallis, MT 59828

IMPACT STATEMENT

Utilizing tests to determine nitrate accumulation in forage is an important tool for producers. This publication outlines the protocols and efficacy of the Nitrate QuikTest, Nitrate Strip Test, and Commercial Laboratory Analysis.

SUMMARY

Nitrate toxicity is a serious concern for many producers in Montana. The large quantity of annual forages grown, coupled with the likelihood of environmental stress during the growing season, increase risk for nitrate accumulation in plants. In order to prevent decreases in animal performance, or even death, an accurate method of nitrate estimation is required. Currently, the most accurate method for nitrate determination is laboratory analysis, however some tests, which are faster and cheaper than commercial analysis, have been developed. Therefore, this study was designed to evaluate two common qualitative nitrate testing methods, the Nitrate Strip Test and the Nitrate QuikTest. The objective of the study was to compare these tests to a laboratory analysis and determine their accuracy in order to provide producers with more nitrate testing options. Compared to commercial testing, the Nitrate Strip Test was more accurate than the Nitrate QuikTest, matching commercial lab analysis results 77% of the time. The Nitrate QuikTest was slightly less accurate, correctly estimating whether nitrates were present 69% of the time. However, the Strip Test had far more false negatives, whereas the QuikTest had far more false positives. Overall, the Nitrate Strip Test appears to be a better method of estimation compared to the Nitrate QuikTest, while commercial lab analysis still remains the gold standard.

INTRODUCTION

All ranches rely on forages such as native range, introduced pasture, or hay to feed their livestock. Many livestock enterprises in Montana use a combination of several types of forages, including some that have the potential to contain toxic levels of nitrates (NO_3^-). Nitrate toxicity associated with feeding forages reduces an animal's ability to transport oxygen in the blood, and can significantly decrease animal performance. Ruminants like cattle, sheep, and goats are more prone to NO_3^- toxicity than non-ruminants such as horses and pigs.

The gold standard for nitrate testing is quantitative testing via a commercial laboratory. However, commercial testing is costly and time-consuming, creating delays in harvest schedule, which can affect forage quality. To help producers minimize delays in their harvest schedule, there is currently a qualitative NO_3^- test available called the Nitrate QuikTest. This test is designed to give a qualitative estimate of nitrate levels in-field, prior to harvest. However, the test can only be administered by trained personnel due to the caustic solution, and is a very subjective test that does not provide any indication of nitrate levels, only presence of nitrates.

A newer test, the Nitrate Strip Test, is now available commercially. This test is similar to a pH strip test where a strip changes color based on the level of nitrates present, and producers can buy the materials online. However, this test has not been verified in-field in Montana. Therefore,

the objective of this research was to evaluate the efficacy of the two tests, the strip test and the QuikTest, and compare their results to a commercial lab analysis.

PROCEDURES

A two-year study was initiated in Montana in 2016. Extension agents from 14 counties throughout Montana volunteered to collect, test, and record samples brought in to their offices. Agents tested all samples that were brought in using the QuikTest, and randomly selected samples to be tested using the strip test. At the request of producer, each agent then verified several of their samples by submission to a commercial testing lab (Midwest Laboratories, Omaha, NE).

All data were recorded over the two field seasons, and were submitted to the MSU Forage Research Lab for analysis. Data were analyzed based on forage species, county of origin, and nitrate test type.

RESULTS AND DISCUSSION

When comparing the Nitrate Quiktest to the commercial lab results, the QuikTest only correctly identified presence of nitrates in 69% of the samples (44/ 64 samples; Figure 1). Of the 31% that were incorrect, 87% of those were false negative, or the Quiktest predicted the forage was safe while the commercial analysis found levels to be high.

The Strip Test did a better job at predicting forage nitrate levels, correctly estimating 73% of the samples (53/ 73 samples) compared to the commercial analyses (Figure 2). Of the 27% that were inaccurate, 45% (12% overall or 9/ 73

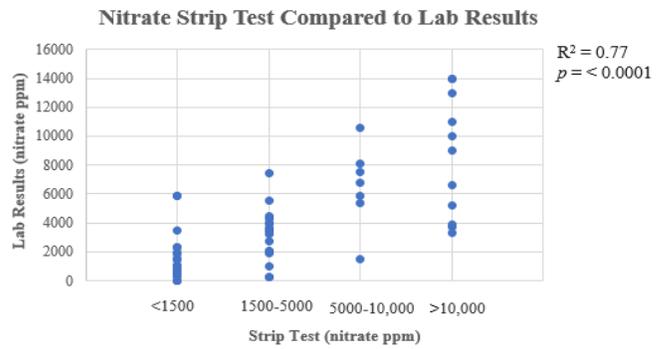


Figure 2. Comparison of nitrate strip test results to commercial lab results.

samples) of those were false positives, or forages that were evaluated by the strip test to be higher than they were in the commercial analysis. The remaining 15% of tests (11/ 20 samples) were false negative.

Due to the uneven number of samples from each species submitted, a thorough statistical analysis of species differences was not possible. However, the largest reported levels from commercial analysis were from oats, weeds, barley, warm-season grass, and surprisingly, silage samples. Care should be taken in interpreting these results, as submissions from those classified as “silage” were limited, and had lower sample numbers compared to other species categories such as oats and barley.

Another interesting result from this study is the comparison between 2016 and 2017 (Figure 3). When all samples were averaged between the two years, 2017 had much higher reported NO₃⁻ levels (averaging approximately 5000 ppm using the strip test) compared to 2016 (averaging around 3250 ppm using the strip test). This is not surprising as 2017 was a much hotter and drier year compared to 2016 for most of Montana, which is a risk factor for elevated NO₃⁻ levels.

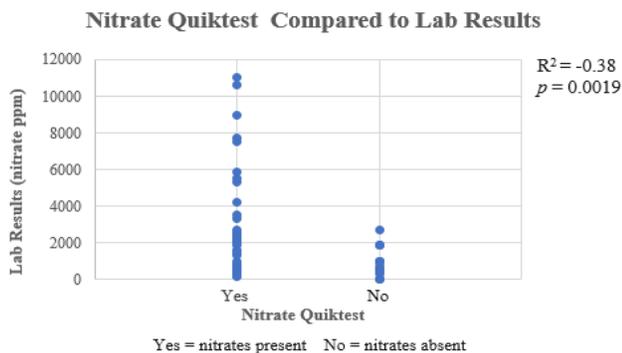


Figure 1. Comparison of Nitrate QuikTest results compared to commercial lab results.

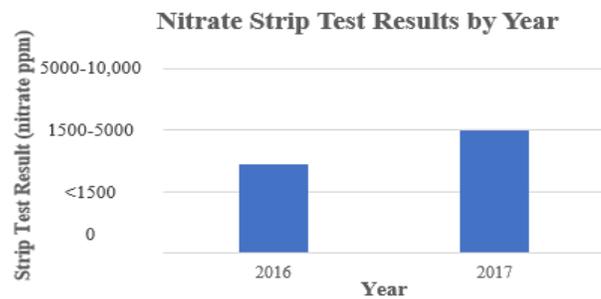


Figure 3. Comparison of nitrate strip test results in 2016 versus 2017.

Overall, authors of this study conclude that the Nitrate Strip Test is an acceptable method of estimating nitrate levels in-field and is more reliable compared to the Nitrate QuikTest. Testing accuracy does depend significantly on the quality of the sample provided, and so with either test, ensure that you are correctly sampling your field or hay stack. However, a laboratory analysis still remains the “gold standard”

whenever nitrate toxicity is a concern in your forages.

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*Corresponding author email:
emily.meccage@montana.edu

Pea Protein: Measurement, Management, and Drought Impact

M. Bestwick^{1†}, P. Miller^{2*}, C. Jones² and K. Olson-Rutz²

¹Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT

IMPACT STATEMENT

Based on yellow pea samples and associated management details from farms in Montana and North Dakota, water limitation may be the most important growing condition that increases pea protein in dryland pulse crop production. The use of granular rather than seed-coat inoculant correlated with higher pea protein under severe drought conditions. Other than a boost in protein with sulfur fertilization in moderate drought condition, starter phosphorus, potassium, and sulfur applications were not correlated with pea protein.

SUMMARY

The study objective was to determine standard management and environmental effects on yellow pea protein, while assessing precision of pea protein measurements. We collected 149 independent yellow pea samples and associated management details from the 2013-2016 growing seasons from 82 pea producers in Montana (71) and North Dakota (11). Observed standard practices for yellow pea included, 1) no-till seeding in cereal stubble in April, 2) use of rhizobia inoculant, and 3) use of starter phosphorus (P), potassium (K), and sulfur (S) fertilizers and pesticides. Protein concentration ranged from 18.9 to 29.6%, with a mean of 23.5%. There was large variation in pea variety, rhizobia inoculant type (granular vs. powder seed-coat), and nutrient management (application of P, K, or S fertilizers). There was higher seed protein in severe drought (24.4%) than wet (22.9%) environments; higher protein (by 1.6 units) when granular inoculant was used compared to peat-powder seed-coatings under severe drought conditions, and higher protein (0.8 units) under moderate drought conditions when S was used. There was no correlation found between application of P or K fertilizers and yellow pea protein, but the lack of response may be due to uncertainty in measuring protein content in yellow pea.

INTRODUCTION

Consumer demand for plant-based protein is rising. If Montana establishes itself as a source of yellow pea with consistently high protein, this will translate directly to greater revenues for Montana pulse growers. This project's primary objective was to identify standard management of yellow pea and identify if management variation affected yellow pea protein across Montana's water-limited growing environments.

PROCEDURES

Eighty-two producers supplied us with 149 yellow pea samples from the 2013-2016 growing seasons along with field management history. We used available climate (Abatzoglou, 2013), soils (Soil Survey Staff-NRCS, 2015), and pea growing degree day models (Miller et al., 2001) to simulate drought stress patterns over the crop cycle. Yellow pea samples were tested at MSU for protein using the combustion method, and protein estimated using NIR (near infrared reflectance). Pea protein measurements were linked to field history to look for protein correlations with growing season management (type of inoculant; P, K, or S fertilizer; pea variety, CDC Meadow vs CDC Treasure) and drought conditions.

RESULTS

Protein Content

The average protein content based on combustion method of these samples was 23.5%,

but protein ranged from 18.9-29.6%. We evaluated both the variation among duplicate samples in the combustion method, and the precision and accuracy of NIR, the main protein industry method for yellow pea protein determination (Bestwick et al. 2018a). The average difference in duplicate combustion was 1.2 protein units. The average difference between NIR estimates and combustion measurements was 1.0 protein unit. Thus, accuracy of protein measurement is a key challenge.

Standard Management

The primary management variables that were *similar* across farms were as follows. Yellow pea is:

- seeded in April
- grown with conventional fertilizer and pesticide inputs
- grown with no-till seeding
- inoculated
- generally seeded following wheat or barley.

Management variables that were *not similar* across farms were as follows:

- Variety selection. CDC Meadow and CDC Treasure made up 52% of the samples, while more than 18 varieties made up the remainder.
- Inoculant. Farms were almost evenly split between using peat-granular (45%) or peat-powder seed-coat (51%) inoculant. Three farms used liquid inoculant, and two farms did not specify if they used inoculant.
- Nutrient management varied across farms. Forty percent of farms did not use fertilizer on yellow pea and 60% used various blends and rates of P, K and S fertilizer. Phosphorus was likely applied as a monoammonium phosphate blend (11-52-0) contributing the low amounts of nitrogen (N) reported (94% reported applying 2 to 11 lb N/acre). Of the 60% of farms that *did* apply fertilizer, the proportion that applied P, K and S, along with ranges in rates, are as follows:
 - P₂O₅: 98%, 15 to 52 lb/acre
 - K₂O: 18%, 5-10 lb/acre
 - S: 61%, 3-8 lb/acre.

Typical Growing Environments

Each line in Fig. 1A represents a simulated “drought stress pattern” for individual pea-field history samples. A detailed description of the mechanics of the simulated drought patterns can be found in Bestwick (2016). These drought stress patterns were grouped into three drought environments interpreted as favorable (green), moderate (yellow), and severe (red; Fig. 1B). In the favorable drought stress environment, pea did not undergo significant drought stress until after flowering. Favorable drought stress was due to timely precipitation, heavy soils, and moderate temperatures or any combination of environmental factors that provided the crop adequate soil water supply. Conversely, in the high drought stress environment, pea was subjected to drought stress beginning in the vegetative (V) growth stages, increasing in reproductive (R) growth stages (after flowering), and lasting to maturity. A high drought stress environment can result from low rainfall, hot temperatures, soils with low water holding capacity, or any combination of environmental factors that limit soil water supply.

Drought Environment and Management on Pea Protein

The highest protein content (24.4%) was associated with severe drought, and the lowest protein content (22.9%) was associated with favorable soil moisture. The impact of drought environment was the same for both CDC Meadow and CDC Treasure, and there were no protein differences between the two varieties. Tao et al. (2017) also found that environment was far more important than variety at driving protein differences, yet found a positive yield – protein relationship suggesting moisture increased protein. The timing and severity of drought influences its impact on pea protein (Tao et al., 2017; Bestwick et al., 2018a), although less predictably than in wheat. Tao et al. (2017) did find differences in how protein in each of nine cultivars responded to growing environment.

In severe drought, granular inoculant produced higher protein (24.5%) than seed-coat inoculant (22.9%). Granular inoculant has been reported to be superior to peat-powder seed-

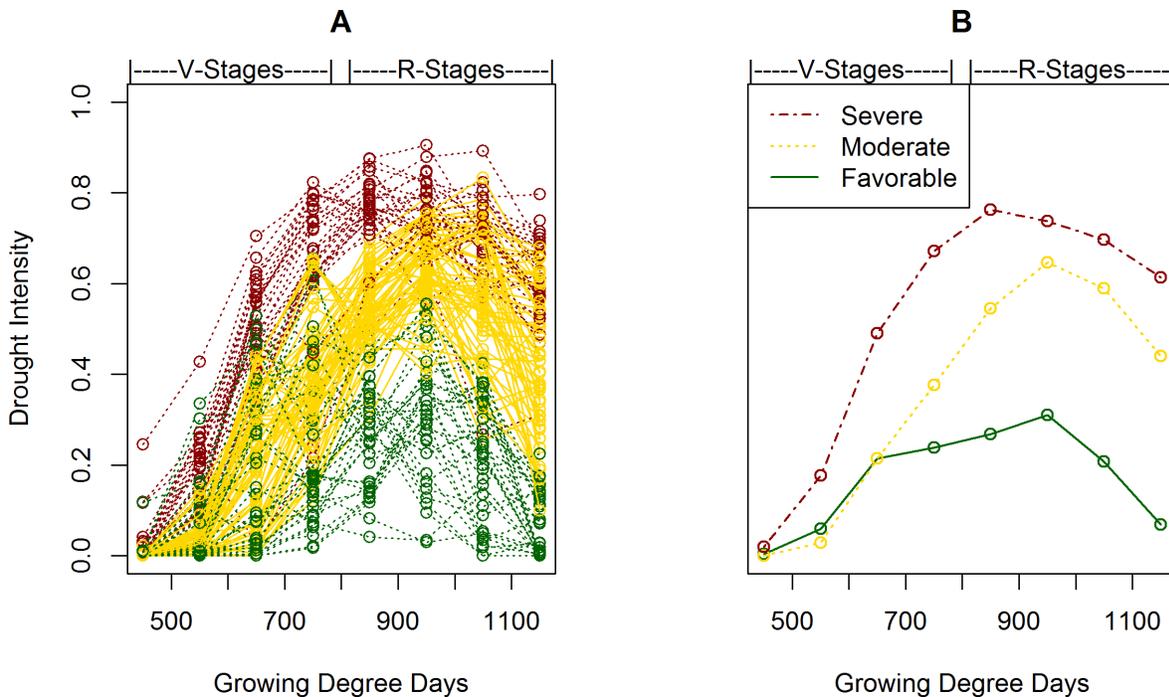


Figure 1. (A) Individual field drought simulations, and (B) typical drought patterns derived from clustering techniques. Drought intensity 0 indicates no drought stress and a value of 1 indicates extreme drought stress. V is vegetative and R is reproductive growth stages.

coatings in dry growing conditions (Clayton et al., 2004), likely because granular promotes nodule formation along the tap and lateral roots rather than clustered near the root crown, where it is often drier.

Sulfur appeared to increase protein in moderately drought stressed environments. Greenhouse studies have shown that N fixation is higher in soils with adequate S (Zhao et al., 1999), but at the time of writing we know of no regional field trials that address the impact of S on N fixation and protein content in pea.

Phosphorus and K fertilization did not correlate with yellow pea protein. After a comprehensive review of protein formation in pea (Bestwick et al., 2018b), these results are expected. Studies throughout the Canadian prairies found that nutrient management can have significant effects on yield, but protein was often unaffected (McKenzie et al., 2001a, b). This could be in part because pea seed and protein formation occur simultaneously, that is, under adverse conditions both the rate of N transferred to the seed and seed number are lower. The uncertainty in pea protein measurements (see

Protein Content above) contributes to the difficulty in finding statistical differences in management and environment impacts on pea protein.

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*Corresponding author email: pmiller@montana.edu

†Current address: Jackola Engineering and Architecture, Kalispell, MT

How Far Do Guard Dogs Roam from their Sheep?

J.C. Mosley^{1*}, R.A. Frost¹, B.L. Roeder², S.L. Wells¹, L.B. McNew¹, and P.E. Clark³

¹Department of Animal and Range Sciences, Montana State University, Bozeman, MT 59717, ²Montana State University Extension, Choteau, MT 59422, and ³USDA Agricultural Research Service, Boise, ID

IMPACT STATEMENT

Keeping range sheep 0.25 mile away from trailheads, campgrounds, or picnic areas during weekends, holidays, or other high potential recreation use periods should prevent most encounters between guard dogs and recreationists. The presence of wolves and grizzly bears on the landscape did not influence how far guard dogs roamed from their sheep.

SUMMARY

Direct and indirect losses from predators combine to make predator control an issue of great importance to US livestock producers. Guard dogs are an economical, non-lethal form of predator control. As such, guard dogs are fundamentally important to sustaining food and fiber production by livestock grazing enterprises in the western US. This is especially true as we progress into a future marked by ever-increasing populations of large carnivores. Strategies are needed to mitigate potential conflicts between guard dogs and humans. We studied guard dog behavior in the presence and absence of wolves and grizzly bears. Our results indicate that wolves and grizzlies did not influence how far guard dogs roam from their sheep. Our results also indicate that range sheep producers do not need to incur extra costs by enlarging ungrazed buffers surrounding high-use recreation sites or rural residential areas. The American Sheep Industry Association currently recommends that sheep and associated guard dogs be kept at least 0.25 mile from any trailhead, campground, or picnic area during weekends, holidays, or other high potential recreational use periods. Our results suggest that keeping range sheep 0.1 mile away from recreation sites will markedly reduce the likelihood of recreationists encountering a guard dog. Keeping range sheep 0.25 mile away from recreation sites will likely prevent most, but not all, encounters between guard dogs and recreationists.

INTRODUCTION

According to the USDA National Agricultural Statistics Service, wild predators killed 20,000 Montana sheep and lambs in 2017, a 19% increase from 2016. Sheep and lambs lost to predators cost Montana sheep producers more than \$3.2 million in 2017.

Guard dogs are a non-lethal tool used by Montana sheep producers to deter depredation and to coexist with wild predators. Guard dogs have been used worldwide for centuries, but widespread use of guard dogs did not begin in the western US until the latter 1970s, after passage of the Endangered Species Act and concurrent restrictions on the use of poisons for lethal predator control. Research in the 1970s and 1980s helped develop management strategies for using guard dogs, and guard dogs were proven effective for protecting livestock (mostly sheep) from predation (mostly by coyotes). Very limited research has occurred with guard dogs since the 1980s, but the situation in the western US has changed dramatically in the past few decades, providing new challenges. Two of the biggest changes have been: 1) the expansion of wolf and grizzly bear populations, and 2) the expansion of human recreation and rural residential development on forest and rangeland landscapes. These two changes have converged to threaten the continued use of guard dogs for protecting livestock from depredation.

Beginning in the 1970s, guard dogs in the western US were selected for their aggressive behavior against predators (mostly coyotes) and non-aggression to humans. Many, if not most, sheep and goat ranchers in the West, and increasing numbers of cattle ranchers, could not continue producing livestock without the use of guard dogs. However, increasing concerns about potential conflicts between guard dogs and human recreationists are threatening the continued use of guard dogs. For example, in a widely publicized incident in Colorado, a mountain biker was attacked by two guard dogs on a public land grazing allotment. The rancher was held liable for having dangerous dogs not under control on federal public land. In response, the American Sheep Industry Association (ASI) developed an initial set of Best Management Practices (BMPs) to help sheep producers optimize the use of their guard dogs while minimizing potential conflicts with neighbors and recreationists. One BMP recommends that sheep and associated guard dogs be kept at least 0.25 mile from any trailhead, campground, or picnic area during weekends, holidays, or other high potential recreational use periods. Prior to our study, no published data existed to support or refute this recommendation.

Our objective was to examine how far, and under what circumstances, guard dogs travel away from the sheep they are guarding. We examined whether guard dog behavior was influenced by season (summer vs. fall), sex (male dogs vs. female dogs), time of day (day vs. night vs. dusk/dawn), moon phase, or whether the landscape was cohabited by wolves and grizzly bears.

PROCEDURES

We placed GPS satellite data collars on a total of 20 guard dogs and 31 sheep during summer (May, June, July, August) or fall (September, October, November, early December) across eight ranches and three years (2012, 2013, and 2014). One band of sheep was studied per ranch, with a typical band comprised of 800 to 1000 mature ewes, 1200 to 1500 lambs, and 400 to 500 yearling ewes. All eight bands were herded, and all eight bands grazed within their traditional

summer-fall grazing areas in foothill and mountain rangeland of western and west-central Montana. Three bands were located in landscapes cohabited by wolves and grizzly bears, and five bands were located in areas of historically low to moderate depredation, mostly by coyotes. At least one guard dog and one sheep was collared per band of sheep. For bands with more than one collared sheep, the sheep with the greatest number of recorded GPS locations was used for data analyses. GPS locations were recorded at 5-minute intervals (24 hours per day) with 16-ft accuracy. Later, we aggregated the data by hour and for each guard dog we calculated its hourly average roaming distance (i.e., the hourly average distance between the guard dog and the GPS-collared sheep within the band of sheep that the guard dog was protecting).

RESULTS AND DISCUSSION

Thirteen of the GPS collars on guard dogs, and 26 of the GPS sheep collars provided usable data, whereas seven GPS collars on guard dogs and five GPS collars on sheep failed to record usable data. Altogether, we analyzed 12,223 hourly average guard dog locations (average = 940 hourly average locations per guard dog) across three years and within seven bands of sheep on seven different ranches. Six of the guard dogs protected sheep bands in landscapes cohabited by wolves and grizzly bears, and seven guard dogs protected sheep bands in areas of historically low to moderate depredation, mostly by coyotes. Nine of the 13 guard dogs were male and four were female.

Overall, roaming distance (i.e., distance between the guard dog and the sheep they were protecting) averaged 0.1 mile. Female guard dogs averaged 272 feet farther from their sheep than male guard dogs, and roaming distance of both sexes averaged 138 feet more during summer than fall. Roaming distance did not differ between guard dogs with or without cohabiting wolves and grizzly bears. Guard dogs roamed 131 feet further from their sheep during a full moon than during a new moon. Guard dogs remained closer to their sheep during mid-day and roamed farther away at night, yet average roaming distance of the 13 guard dogs never

exceeded 0.2 mile during any hour of the day or night, with or without a full moon, in either summer or fall. However, the maximum roaming distance recorded of every guard dog exceeded 0.25 mile. Maximum roaming distances varied widely among the 13 guard dogs, ranging from 0.3 mile to 1.2 miles.

All guard dogs stayed relatively close to their sheep, with 75% of guard dog locations within 0.1 mile of their sheep. Only 8% of all guard dog-sheep distances were greater than the 0.25 mile distance that ASI recommends that sheep and associated guard dogs be kept away from trailheads, campgrounds, or picnic areas during weekends, holidays, or other high potential recreational use periods.

Although roaming distances differed between male and female dogs, day vs. night, and full vs. new moons, the differences were slight and may not be meaningful to sheep producers operating on large landscapes. If possible, however, it may be wise to keep sheep further away from recreational sites and rural residential areas when using female guard dogs and at night, especially when the moon is bright. Roaming distances did vary widely among individual dogs, suggesting that sheep producers may wish to purposely select guard dogs that stay closer to their sheep if their sheep regularly graze near recreational sites or near rural residential areas.

ACKNOWLEDGMENTS

This project was a collaborative effort among seven private ranches, three state livestock producer associations (Montana Wool Growers Association, Montana Public Lands Council, Montana Association of State Grazing Districts), five federal government agencies (USDA-APHIS Wildlife Services, USDA-Agricultural Research Service, US Forest Service, Bureau of Land Management, USDA Natural Resources Conservation Service), the Montana Agricultural Experiment Station, and Montana State University Extension. Special thanks to John and Nina Baucus, Jay Bodner, Bob Brekke, Austin Cantwell, Kevin Halverson, John Helle, Kelly Ingalls, John Lehfeltdt, Jim Murphy, Jon Siddoway, Sven Svenson, John Steuber, Floyd Thompson, Randy Tunby, and Jim Wickel. Funding was provided by the Bair Ranch Foundation and the USDA National Institute of Food and Agriculture–Western SARE (Sustainable Agriculture Research and Education) Grants Program.

*Corresponding author email:
jmosley@montana.edu

Factors Affecting Ewe Somatic Cell Count and Its Relationship with Lamb Weaning Weight in Extensively Managed Flocks

T. W. Murphy^{1*}, W. C. Stewart², and J. B. Taylor³

¹Department of Animal and Range Sciences, Montana State University, Bozeman 59717, ²Department of Animal Science, University of Wyoming, Laramie 82071, and ³USDA-ARS, Range Sheep Production Efficiency Research Unit, U.S. Sheep Experiment Station, Dubois, ID 83423

IMPACT STATEMENT

Clinical mastitis contributes to increased culling rates and lost revenue in Western sheep production, but the importance of subclinical mastitis is less clear. Results from this work suggest that subclinical mastitis is prevalent and has large economic consequences in the form of reduced lamb weaning weight. Strategic trace mineral supplementation and other management practices to reduce its prevalence warrants further investigation.

SUMMARY

The economic impact of mastitis in dairy species is well documented and efforts to reduce its prevalence through husbandry and genetic selection have been extensive. Females from non-dairy breeds are also prone to mastitis but since their udders are not regularly handled and their milk is not a commodity, its prevalence and impact are less clear. Milk was collected from healthy ewes at MSU and the U.S. Sheep Experiment Station (USSES) and somatic cell count was quantified and transformed to the log₁₀ scale (LSCC). MSU milk was collected at approximately 5 (early) and 35 d (peak) after lambing, but only at peak lactation at USSES. Total litter weaning weight at 120 d (LWW) was analyzed separately for each flock. At MSU, early LSCC negatively affected LWW ($P < 0.01$). Peak LSCC reduced LWW at USSES ($P < 0.01$) but not at MSU ($P = 0.87$). On average, a unit increase in early or peak LSCC was associated with a 28.2 and 32.6 lb decrease in LWW at MSU and USSES, respectively. Ewes at MSU that maintained low LSCC (< 5.7) at both collections had greater ($P = 0.02$) serum Zn concentration ($1.53 \mu\text{g} \cdot \text{mL}^{-1}$) than ewes with low LSCC in early and high LSCC (≥ 5.7) in peak lactation ($1.13 \mu\text{g} \cdot \text{mL}^{-1}$). Overall, the percentage of ewes with high LSCC was 19 and 17% at MSU and USSES, respectively.

INTRODUCTION

Mastitis, an inflammation of the mammary gland caused by bacterial infection, is characterized by palpable lumps in the udder, abnormal milk, and tissue discoloration in its clinical state (Menziez and Ramanan, 2001). The average incidence of clinical mastitis (CM) is relatively low (1.2 to 3%) across flocks sampled around the world (Quinlivan, 1968; Arsenault et al., 2008; Cooper et al., 2016), but can have large variation within flocks (0 – 37%; Grant et al., 2016). Nevertheless, CM was the primary reason for culling 6.7% of ewes in the U.S. in 2011 (USDA APHIS, 2011).

Subclinical mastitis (SCM) has no visual symptoms, but can be diagnosed through bacterial culture and/or quantifying somatic cell count (SCC) in milk. The morbidity rate of SCM in sheep is much greater than CM (12 – 50%; Watkins et al., 1991; Keisler et al., 1992). Lambs reared by ewes with experimentally (Fthenakis and Jones, 1990) and naturally acquired SCM (Moroni et al., 2007) have reduced growth. However, the direct effect of maternal SCC on lamb growth has been inconsistent (Ahmad et al., 1992) or insignificant (Gross et al., 1978; Keisler et al., 1992) in the reviewed literature. The objectives of the present study were to identify biological factors that affect ewe SCC and to

quantify the relationship between maternal SCC and lamb growth.

PROCEDURES

The MSU Agricultural Animal Care and Use Committee and the U.S. Sheep Experiment Station (USSES) Institutional Animal Care and Use Committee approved all husbandry practices and experimental procedures used in this study.

Data Collection

Milk was sampled from ewes free from CM at both MSU and the USSES during the spring of 2017. The same Rambouillet ($n = 26$) and Targhee ($n = 30$) ewes at MSU were sampled twice, the first shortly after parturition (< 5 d in milk; early) and the second before turnout to summer grazing (30 to 45 d post-lambing; peak). Milk was collected once from Suffolk ($n = 38$) and crossbred (3/8 Suffolk, 3/8 Columbia, 1/4 Texel; $n = 46$) USSES ewes at peak lactation.

Milk SCC was quantified within 3 d of collection. Blood was collected from MSU ewes into trace element vacutainer tubes and centrifuged. The resulting serum was later analyzed for trace mineral concentration.

Statistical Analyses

Milk SCC was transformed to the \log_{10} scale (LSCC). Ewe LSCC within collection date and flock was analyzed with effects of ewe age [1 (USSES only), 2, or 3+], breed, and number of lambs born (NLB; 1 or 2+). A subset of MSU ewes with low LSCC (< 5.7) at both collections (LL; $n = 12$) and low LSCC at early and high LSCC_A (≥ 5.7) at peak lactation (LH; $n = 12$) were identified. Serum Se and Zn concentrations were analyzed for this subset with fixed effects of LSCC_A class (LL or LH) and collection date and the random effect of ewe.

Lamb weaning weights were adjusted to 120 d and summed within ewe (LWW). Total litter weaning weight at MSU was analyzed in 2 separate models that fit either early or peak LSCC as a linear covariate and the fixed class effects of ewe breed and age. The same model was used to analyze LWW at USSES, but only peak LSCC was available for these ewes.

RESULTS AND DISCUSSION

Production and biological factors influencing ewe SCC

Least-squares means of the main effects of collection date and LSCC group on MSU ewe serum Se and Zn concentrations are displayed in Table 1. Serum Se was $23.4 \text{ ng} \cdot \text{mL}^{-1}$ greater ($P < 0.01$) in peak lactation, but no difference in serum Zn was detected ($P = 0.29$) between collection dates. Early and peak lactation LSCC class had no effect ($P = 0.39$) on serum Se concentration. However, LL ewes had $0.40 \text{ } \mu\text{g} \cdot \text{mL}^{-1}$ greater ($P = 0.02$) serum Zn concentration than LH ewes. Zinc is involved in the production and maintenance of keratinized tissues (O'Rourke, 2009) and Saianda et al. (2007) reported that bacterial adherence to the mammary epithelium was greatly reduced in dairy ewes supplemented with additional Zn.

Ewe age, breed, and NLB did not affect ($P \geq 0.13$) any ewe LSCC measure at MSU or USSES. Waage and Vatn (2008) reported that ewes rearing 3 or more lambs were 6.7 times more likely to develop CM than ewes rearing a single lamb. Additionally, Gross et al. (1978), Ahmad et al. (1992), and Arsenault et al. (2008) reported that SCC increased with ewe age.

Maternal SCC and lamb growth

Least-squares means for the main effects of ewe age and breed and solutions for LSCC on LWW are displayed in Table 2. Not surprisingly, LWW was $14.7 - 59.1$ lbs greater ($P \leq 0.03$) in multiparous ewes than primiparous ewes at MSU

Table 1. Least-squares means (\pm SE) for the main effects of collection date and LSCC class on MSU ewe serum Se and Zn concentrations.

Effect	Level	Serum Trace Mineral	
		Se, $\text{ng} \cdot \text{mL}^{-1}$	Zn, $\mu\text{g} \cdot \text{mL}^{-1}$
Collection date ¹	Early	118.7 ± 4.71^b	1.24 ± 0.12
	Peak	142.1 ± 4.63^a	1.42 ± 0.11
LSCC class ²	LL	127.0 ± 5.59	1.53 ± 0.11^a
	LH	133.9 ± 5.67	1.13 ± 0.12^b

¹Early, Peak = serum trace mineral concentration quantified at early (< 5 d) or peak (30 to 45 d) lactation, respectively.

²LL = ewes with low LSCC (< 5.7) at both early and peak lactation; LH = ewes with low LSCC at early and high LSCC (≥ 5.7) at peak lactation.

^{a,b}Means within an effect and column with no superscript in common are different ($P < 0.05$).

at USSES. Rambouillet and Targhee ewes had similar ($P = 0.88$) LWW at MSU but USSES crossbred ewes weaned 27.1 lbs heavier litters ($P < 0.01$) than Suffolk ewes.

At MSU, LWW was negatively affected by LSCC in early (-28.2 lbs; $P < 0.01$) but not peak lactation ($P = 0.87$). However, peak LSCC reduced LWW (-32.6 lbs; $P < 0.01$) at USSES. According to these estimates, a ewe with a SCC of 1,000,000 cells mL⁻¹ (LSCC = 6.0) is expected to wean 28.2 and 32.6 lbs less total lamb than a ewe with a SCC of 100,000 cells mL⁻¹ (LSCC = 5.0) at MSU (early LSCC) and USSES (peak LSCC), respectively. In a controlled experiment, Fthenakis and Jones (1990) found that ewes induced with SCM produced 37% less milk than healthy ewes and their lambs weighed 8.8% less and consumed 25% more supplemental feed.

The SCC threshold which diagnoses SCM in sheep is not well-defined, with suggested cutoffs varying from 300,000 to 1,000,000 cells • mL⁻¹ (Fthenakis et al., 1991; González-Rodríguez et al., 1995). The percentage of ewes with SCC > 500,000 cells • mL⁻¹ was 19 and 17% at MSU and USSES, respectively. On average, such ewes weaned 14.1 and 48.9 lbs less lamb, a lost revenue of \$31 and \$106 per ewe (\$2.17 lb⁻¹ feeder lamb; USDA, 2018). While mean serum Zn concentration of LH ewes was considered adequate (Herdt et al., 2000), reliance upon Zn deficient forages in the months leading up to

parturition and after weaning may predispose ewes to intramammary infection.

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Table 2. Least-squares means (\pm SE) for the main effects of ewe age and breed and solutions for LSCC on LWW at MSU and USSES.

Effect	Level	LWW	LWW USSES,
		MSU, lbs	lbs
Ewe age, yr	1	-	72.3 \pm 12.0 ^b
	2	77.2 \pm 4.59 ^b	111.6 \pm 10.5 ^a
	3+	91.9 \pm 4.59 ^a	131.4 \pm 5.97 ^a
MSU/USSES breed ¹	R/C	85.1 \pm 4.98	118.6 \pm 7.19 ^a
	T/S	84.0 \pm 4.25	91.5 \pm 7.47 ^b
LSCC ²	Early	-28.2 \pm 8.81 [*]	-
	Peak	ns ³	-32.6 \pm 11.5 [*]

¹Rambouillet (R) and Targhee (T) ewes were sampled at MSU, Crossbred (C; 3/8 Suffolk, 3/8 Columbia, 1/4 Texel) and Suffolk (S) ewes were sampled at USSES.

²Early, Peak = LSCC quantified at early (< 5 d) and peak (30 to 45 d) lactation.

³ns = coefficient is not different from zero ($P = 0.87$).

^{a,b}Means within an effect and column with no superscript in common are different ($P < 0.05$).

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*Corresponding author email:

Thomas.murphy12@montana.edu

Beaver-Mediated Riparian Restoration: Understanding Habitat Conditions Associated with New Colonies

T. D. Ritter and L. B. McNew*

Department of Animal and Range Sciences, Montana State University, Bozeman, MT

IMPACT STATEMENT

Beaver activity provides benefits to stream systems including water storage, sediment retention, and enhanced wildlife habitat. Beavers have therefore become a popular tool for riparian restoration. We studied habitat selection by beavers to provide information for selecting restoration sites that will have the highest probability of colonization.

SUMMARY

The activities of beavers provide a variety of benefits to stream systems by capturing and storing water, retaining sediment, and increasing habitat diversity. Because of these benefits, stream restoration projects that use beavers as a tool to achieve project goals are increasing in popularity. However, there is a need to better understand habitat selection by beavers in unmodified and semi-degraded stream systems that are commonly targeted for restoration projects. We conducted beaver-use surveys in headwater streams in southwest Montana to investigate settlement site selection by beavers colonizing novel areas. Our objective was to evaluate beaver settlement site selection in the context of beaver restoration to improve identification of suitable project locations. We found that habitat conditions influenced settlement site selection at multiple spatial scales. New settlement sites were characterized by low gradients, dense woody riparian vegetation, relatively narrow channels, and variation in stream channel structure. Generally, beavers selected for pre-engineered habitats over unoccupied stream segments, but in relatively unmodified habitats conditions that facilitated stable dam construction appeared to be most important. We suggest managers evaluate habitat suitability at multiple spatial scales when selecting project locations. Additionally, the use of pre-engineered dams and lodges will likely increase the probability of occupancy by beavers.

INTRODUCTION

Beavers (*Castor* spp.) exert profound influence on the habitats they occupy through the construction of dams, digging of channels, and redistribution of woody vegetation. While the effects of beavers occupying an area can vary, beaver activities in smaller headwater stream systems enhance and maintain healthy riparian habitats (Naiman et al. 1988). Beaver dams are particularly beneficial to stream systems both for wildlife habitat and ecosystem services. Beaver dams: 1) buffer against runoff events which protects the stream system from flood damage (Meentemeyer and Butler 1999), 2) capture and store precipitation which can augment low flows in the summer and fall (Naiman et al. 1988, Westbrook et al. 2006), and 3) increase retention of sediments which can expand riparian areas and reconnect streams to their floodplains (Gurnell 1998, Meentemeyer and Butler 1999, Pollock et al. 2014).

Due to the wide range of potential benefits, beavers are increasingly used as a tool for stream and riparian habitat restoration (Pollock et al. 2018). Beaver restoration projects are especially common in the western United States where water conservation and riparian habitat enhancement are prominent objectives for human use and ecosystem health (Pilliod et al. 2017). Because beavers can be effective agents of stream restoration, managers generally want to use them in semi-degraded streams that represent

suboptimal habitat. However, the selection of restoration sites is typically based on an understanding of beaver habitat suitability that may not accurately reflect the selection process in suboptimal habitats. Beavers drastically modify their surroundings which fundamentally alters important information about habitat conditions that promoted colonization. Yet, most beaver habitat suitability studies compare habitat at well-established colonies to unoccupied or abandoned stream sections (Beier and Barrett 1987, Barnes and Mallik 1997, Suzuki and McComb 1998). As a result, many metrics used to assess suitable habitat may be altered by the time researchers collect data, and therefore may not accurately portray the state of the habitat when the colony was first established.

We conducted beaver-use surveys on willow-dominated, mountainous tributary streams in the upper Gallatin and Madison River drainages in southwest Montana to investigate habitat selection by beavers when they are colonizing suboptimal habitats that are relatively unmodified by beaver activity. Our primary research objectives were to: 1) map stream sections that were relatively unmodified by beavers, 2) identify new settlement sites in unmodified habitats, 3) compare multi-scale habitat conditions at new settlement sites with unsettled sites to identify factors associated with colonization, and 4) provide baseline information for evaluation of beaver habitat suitability to identify restoration sites with the highest probability of colony establishment.

PROCEDURES

Beaver-use surveys were conducted during July–November, 2015–2017. We walked along streams in the study area and marked all active and inactive beaver sign including dams, lodges, food caches, castor mounds, and foraging areas. We then used the spatial distribution and relative age of sign to classify 1/4-mile stream segments as active, abandoned, relic, or unoccupied. Active stream segments were part of established beaver colonies. Abandoned stream segments had lodges and dams in place but the structures were not being actively maintained by beavers. Relic stream segments had sign of past beaver

occupancy, but dams and lodges would need to be completely rebuilt for beavers to occupy the area. Unoccupied stream segments contained no sign beavers ever had a well-established colony in the area. We defined new settlement sites as any beaver colony that was established within two years of the survey and was located in a stream segment previously considered relic or unoccupied.

We measured habitat conditions representing three categories hypothesized to be important to beaver habitat selection: stream geomorphology, woody vegetation resources, and wetland types. We evaluated habitat at two spatial scales. First, we measured habitat conditions using remote-sensing data and a GIS to represent a broad-scale habitat suitability assessment. Second, we measured more fine-scale habitat conditions in the field.

We used logistic regression to compare newly-settled stream segments to unsettled stream segments. We only considered stream segments that were classified as relic or unoccupied for at least one year of the study as available to be newly settled. Therefore, abandoned and active segments were not included in the analysis. We constructed models using combinations of habitat variables we hypothesized would have the greatest impact on the probability a stream segment would be newly settled by beavers.

RESULTS AND DISCUSSION

We surveyed 152 miles of 27 streams in the study area over three years. Within these streams, we surveyed 613 stream segments that were 1/4 mile in length; 370 of which were considered relic or unoccupied for at least one year of the study and were therefore available to be newly settled. Over the study period we identified 27 new settlement sites which accounted for 48 of the 370 available stream segments (13%). Thirty (63%) of the settled segments were classified as relic and 18 (37%) were classified as unoccupied. Thus, beavers apparently selected for pre-engineered settlement sites even if the sites did not provide pre-built dams and lodges. Although not included in our analysis, we noted abandoned stream segments were settled at a high rate,

providing further evidence beavers selected for pre-engineered habitats. The reasons for the selection of pre-engineered habitat is unclear, but it is possible the presence of old dams and lodges is enticing to beavers because they do not have to spend as much time and energy building new structures. Alternatively, beaver activity may improve habitat conditions that encourage future occupancy regardless of previous infrastructure. The fact that relic and abandoned stream segments were settled at a similar rate suggests habitat changes brought about by beaver occupancy may be more important than pre-built dams and lodges.

Beavers selected stream segments with low gradients, dense woody riparian vegetation, narrow stream channels, and low-lying areas next to the stream. Field-based habitat sampling suggested settled segments had greater variation in channel width and depth, and had greater channel complexity in the form of side channels, backwaters, and tributaries. Our results suggest beavers selected for new settlement sites that facilitated stable and efficient dam construction. Low gradient streams reduce stream power acting on the front of the dam (Pollock et al. 2014) and narrower streams may be easier to dam (Suzuki and McComb 1998). Low-lying areas next to the stream may provide a larger flooded area behind the dams and may relieve pressure on the dam during high water periods as water is able to escape the channel into the floodplain. Greater channel complexity and variation in channel form likely provide a variety of microsites to fulfill the requirements of colony establishment (Scrafford et al. 2018). Our field observations indicated most new settlement sites were formed around distinct pools of deeper water next to undercut banks where the initial lodge was located. The first dam was then built at the tail-out of the pool where the water became shallow. By following this pattern, beavers could build a smaller dam to meet their needs while also taking advantage of side channels, midstream rocks, or sandbars that provided critical anchor points for the new dam.

When evaluating potential locations for beaver restoration projects, we suggest managers evaluate habitat suitability at multiple spatial

scales. An initial screening of the project area using a GIS and aerial imagery can give managers an overview of suitable habitat, which can then be refined using field-based habitat assessments. Stream segments with previous beaver modifications may be highly sought-after by beavers, even if modifications are old and are no longer directly influencing the stream channel. The selection for modified habitats indicates construction of rudimentary dams and lodges at a restoration site may be a necessary step to encourage beaver occupancy. Whenever possible, such structures should be built in stream segments with characteristics similar to those described by our habitat selection models, with greater channel complexity, more variation in stream channel form, and conditions that can facilitate stable dam construction.

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*Corresponding author email:

lance.mcnew@montana.edu

Molecular Pathways for Muscle and Adipose Tissue Are Altered Between Animals Classed as Choice or Standard

S. A. Blatter, J.A. Boles, J.M. Thomson* and J.G. Berardinelli

Department of Animal and Range Sciences, Montana State University, Bozeman, MT, 59717

IMPACT STATEMENT

Beef tenderness is the driving force behind consumers decisions to consume beef. When the eating experience is poor or inconsistent, genetic improvement can be one strategy to improve meat eating experience. Identification of patterns of gene expression in muscle from carcasses of different quality grades allows a better understanding of the metabolic changes that occur as fat deposition increases both intramuscularly and in adipose depots and will provide targets for genetic improvement and increased tenderness and consistency.

SUMMARY

Genetic improvement of beef product quality benefits both producers and consumers. Beef tenderness is a leading influence in consumer decisions to consume beef. Insufficient marbling and subsequent lower quality grades, lack of a uniform product, reduced or insufficient tenderness, high carcass weight or high yield grade can negatively impact beef acceptability. Improvements can be achieved in many of these characteristics by making selection decisions based on the results of genetic evaluations in the form of EPDs, real-time ultrasound imaging, and physical evaluation of candidate breeding animals. The gene expression profiles of muscle and adipose tissues in Standard and Choice carcasses was evaluated. Gene expression differences were profiled to determine the altered metabolic pathways to increase our understanding of the changes that occur in these tissues with increasing fat deposition. This can be used to identify genetic improvement targets to increase product quality and consistency.

INTRODUCTION

Intramuscular fat has been used for quality assurance systems within the US and Australia. Marbling at high levels have been related to tenderness. Tenderness has been identified as the most important factor affecting a consumer perception of palatability of meat. However,

Platter et al. (2003) found that when a trained tasting panel evaluated strip loin steaks from different USDA Quality grade categories, they reported significant variation in tenderness was within a category of quality grades. This suggests that quality grade alone will not guarantee tenderness. As an animal approaches maturity it exhibits significant changes in its physiology. These changes encourage the deposition of fat, including marbling. Thus, there is a need to identify what changes occur at the molecular level that initiates fat deposition that are related to tenderness.

While advances have been made with regards to genetics, and the influence they have on meat tenderness, the question of what specific genes and how these genes function to determine meat quality remains. Previous work at MSU (Engle, 2015) utilized a cohort of Hereford steers that produced carcasses grading Standard, Select and Choice. *Longissimus lumborum* muscle was sampled at slaughter and a significant number of differences were observed between Choice and Standard carcass pools (1,258 genes < 0.01). A functional analysis, was run using DAVID bioinformatics software, which revealed differences in the underlying pathways regulating muscle cell growth and proliferation. Biological processes such as growth, muscle hypertrophy, protein kinase activity, and the lipid biosynthetic pathway were found to be enriched

in the differentially expressed gene set. The objective of this work was to provide new insight into the molecular and genetic basis of meat quality grade.

PROCEDURES

Data collections from these animals were in compliance with the Montana State University Agriculture Animal Care and Use Committee (Protocol No. 2015-AA17).

Fifteen steers, born at the Red Bluff Research Ranch, Norris, MT, were selected at weaning based on weight and date of birth, the selection parameters were: ± 22.7 kg and birthdates within a 14-day window. Steers were relocated to the Montana State University Bozeman Area Research and Teaching Farm and placed in a single pen in the feedlot. At the start of the study, steers weighed an average of 315 kg. Each steer received a Synovex One Feedlot implant per standard feedlot protocol. Steers were fed an ad libitum standard feedlot diet and had free access to water. Steers were randomly allocated to one of three endpoints based on body weight, with average endpoint weights of 431 kg, 522 kg, and 612 kg for Standard, Select, and Choice quality grades, respectively. These weight-based endpoints achieved the desired marbling endpoints reported in Table 1.

Steers were harvested following normal harvest procedures utilizing a mechanical stun. Intermuscular and subcutaneous adipose tissue samples were taken at time of harvest and homogenized immediately. *Longissimus thoracis* muscle samples were snap-frozen in liquid N₂ for later gene expression analysis. Twenty-four hours after slaughter, carcass data were collected by trained personnel. The decline in pH of each carcass was monitored in the *longissimus lumborum* muscle approximately 6 cm from the hip bone using an Accumet Portable AP110 meter, (Fisher Scientific) equipped with an Orion 8163BNWP electrode. Measurements were taken hourly up to 8 h, then at 24 h postmortem to measure 'ultimate' pH.

RNA Extraction and quantification

Frozen muscle samples and homogenized intramuscular and intermuscular adipose tissue

samples RNA was extracted using a Qiagen RNeasy Plus Universal Midi kit according to manufacturer recommendations. Extracted RNA was stored in a -80° C freezer. Due to RNA degradation in some samples we were unable to utilize samples from each adipose depot from each animal. The highest quality RNA sample from each animal was sent for further analysis, as well as sending random samples from each depot for 3 samples to verify that the adipose samples, regardless of depot, had a sample correlation of greater than 0.80.

RNA sequencing and quantification

Three μ g of RNA per sample was used for library preparation. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample. RNA libraries were sequenced and then mapped to the bovine reference genome UMD 3.1. An index of the reference genome was built using Bowtie v2.2.3 and paired-end clean reads were aligned to the reference genome using TopHat v2.0.12. The use of HTSeq v0.6.1 was employed to count the read numbers mapped to each gene. The FPKM, expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced of each gene was then calculated based on the length of the gene and the reads count mapped to said gene.

Differential expression analysis

Prior to differential gene expression analysis, data was normalized. Differential expression was analyzed for two conditions and the p-values were adjusted using the Benjamini-Hochberg method. This was done to minimize false positives and to ensure the observed differences were real.

GO and KEGG enrichment analysis

Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the Goseq R package, in which gene length bias was corrected. GO terms with FDR corrected p-values of less than 0.05 were

considered significantly enriched by differentially expressed genes. KEGG, a database resource for understanding high-level functions and utilities of the biological system was used, along with KOBAS software, to test the statistical enrichment of differentially expressed genes in KEGG pathways.

RESULTS AND DISCUSSION

Carcass weight from animals classified in the Choice quality grade were greater ($P = 0.002$) than carcass weights from animals classified as Select or Standard (Table 1). Furthermore, fat thickness was greater ($P = 0.007$) for carcasses from animals classified as Choice and Select than from animals classified as Standard. The use of the weights as endpoints did place most of the carcasses in the correct grade categories as indicated by the significant differences in marbling for each category. In contrast, there was no difference in ribeye area between the different categories, indicating that muscle growth had slowed even at the lower fat categories.

Table 1. Carcass characteristics of steaks from carcasses classified as choice, select and standard

	Choice	Select	Standard
Hot carcass wt (lb)	748 ^a	608 ^b	536 ^b
Fat thickness (in)	0.55 ^a	0.39 ^a	0.20 ^b
Ribeye area (in ²)	11.0	9.9	10.4
Marbling score ^y	510 ^a	382 ^b	285 ^c

^{a-c}Means within a row with differing superscripts are significantly different ($P \leq 0.05$)

^yMarbling scores: 200 = traces, 300 = slight, 400 = small, 500 = modest, 600 = moderate.

^zN= 9.81 kg

KEGG pathway enrichment

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a collection of manually curated databases dealing with genomes, biological pathways, diseases, drugs and chemical substances. Pathway enrichment analysis identifies significantly enriched metabolic pathways of signal transduction pathways

associated with DEGs compared with the whole genome background. The greatest differences in pathway enrichment was seen in adipose and muscle tissues between the carcasses classified as Choice and Standard. Thus, these differences are the focus in this report.

In the comparison between muscle from Standard and Choice carcasses, 15 genes were down regulated and 20 were up regulated. The insulin receptor substrate 1 (IRS 1) gene was the only known functionally important gene to be differentially expressed. Interestingly, there was an increased expression of Major Histocompatibility Complex genes in muscle, generally indicating a sign of inflammation or increased macrophage migration to the tissue in fattening animals. Alternatively, this could mean an altered stress response.

There were 49 down-regulated genes and 113 up-regulated genes in the comparison between adipose tissue from Standard and Choice carcasses. Upregulated genes included: CAB39L, FGF-1, GRIN1, LEP, HK2, YWHAG, ACC1, SCD1 and ELOVL3. These genes are mostly related to the metabolism of fat and energy. For example, up-regulation of ACC1, HK2, and the down-regulation of EIF43BP1 would inhibit protein synthesis and promote lipogenesis and glycolysis. This suggests a change from actively growing muscle to depositing fat in Steers that graded Choice. Furthermore, up regulation of leptin which can cause the inhibition of insulin resistance, and the down regulation of gluconeogenesis, which can contribute to altered fat metabolism.

In conclusion, many changes were observed in gene expression in adipose and muscle tissues between steers that were classified as Choice or Standard. KEGG pathway enrichment indicated some overlap and interaction between the different genes that would tend to have contradictory responses in the tissue. However, organisms are complex systems, with metabolic processes under multiple levels of regulation, so considerably more research is necessary to clarify what these changes mean to determining and predicting quality grade in cattle.

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*Corresponding author email:
jennifer.thomson@montana.edu

Effects of Livestock Grazing Management on Occupancy of Mesocarnivores in a Northern Mixed-Grass Prairie Ecosystem

S.T. Vold, and L.B. McNew*

Department of Animal and Range Sciences, Montana State University, Bozeman, MT

IMPACT STATEMENT

Livestock grazing management practices that influence the occupancy of mesocarnivores should be considered for their potential indirect effects on prey populations such as ground-nesting grassland birds, a guild of recent conservation concern.

SUMMARY

Mesocarnivores have recently experienced population increases and range expansions as a result of anthropogenic extirpation of top predators in grassland ecosystems. Mesocarnivores are often major predators of grassland birds and their nests, so considering the effects of management actions on mesocarnivore occupancy is important for grassland bird conservation efforts. Our study evaluates the relative effects of livestock grazing management on mesocarnivore occupancy in a northern mixed-grass prairie ecosystem of eastern Montana. During 2016–17, we deployed camera traps at 90 locations within pastures managed under rest-rotation grazing systems, and at 90 locations within pastures managed under season-long stocking or summer rotational grazing systems. We found occupancy of mesocarnivores was highest in rest-rotation grazing systems, followed by season-long and summer-rotation systems, respectively, and showed a positive relationship with stocking rate.

INTRODUCTION

The extirpation or acute population reductions of top predators throughout much of their historic ranges in North America has resulted in increased abundances and range expansions of secondary predators (hereafter mesocarnivores), an ecological phenomenon known as “mesopredator release” (Crooks and

Soulé, 1999; Prugh et al., 2009; Brashares et al., 2010). Expansion of mesocarnivore populations has increased pressure on their prey base within numerous ecosystems throughout North America, and has been attributed to ecosystem instability, along with population declines of birds, small mammals, and reptiles (Ritchie and Johnson, 2009; Brashares et al., 2010). Mesocarnivores can have a substantial impact on the annual fecundity of ground-nesting birds in grassland ecosystems through depredation of eggs and young at nest sites while foraging, as well as predation of fledglings and adults while hunting (Vickery et al., 1992; Rollins and Carroll, 2001; Hovick et al., 2011).

Grassland birds have undergone steep population declines throughout a majority of their historic range over the past century (Knopf, 1996; Peterjohn and Sauer, 1999; Sauer et al., 2013). Rangelands managed for livestock grazing contribute the majority of remaining native grassland bird habitat (Knopf, 1996; Davis et al., 2014). However, researchers have suggested that even large areas of native grassland habitat may not be adequate to sustain grassland bird populations in light of recent population trends (With et al., 2008). Although lowering rates of nest predation should benefit grassland bird conservation efforts (Johnson et al., 2012), research evaluating the effects of livestock grazing or rangeland management practices on grassland bird nest predators in prairie ecosystems is lacking. Researchers have hypothesized that livestock grazing increases

predator activity (Fuller and Gough 1999), and have found occupancy of certain mesocarnivores is higher in grazed than ungrazed grasslands (Gese and Thompson 2014). However, few studies have evaluated the direct effects of livestock grazing management on mesocarnivore occupancy.

PROCEDURES

Our study area was located within the northern mixed-grass prairie and centered on a ~3,000-ha Upland Gamebird Enhancement Program (UGBEP) project established by the Montana Department of Fish, Wildlife, and Parks, in eastern Richland County, Montana, 20 km southeast of Sidney (48° 30' N, 104° 04' W). The UGBEP project was managed under a 3-year, 3-pasture rest-rotation grazing system. Five pastures of ~4,300-ha bordering the UGBEP project were used as reference treatments, with three pastures located on private lands adjacent to the UGBEP project in Montana, and two pastures located on U.S. Forest Service lands adjacent to the UGBEP project in McKenzie County, North Dakota. The reference pastures were managed under season-long grazing or 2-pasture summer-rotation grazing systems. We interviewed landowners to gather grazing system data, as well as cattle turn-out/turn-in dates and number of head stocked to calculate stocking rates for the pastures within the study area.

We collected data during two field seasons (May – July, 2016–17). We randomly generated 180 camera trap survey sites (90 each season) across gradients of habitat conditions within the UGBEP project and reference pastures. We deployed one passive infrared remote field camera (Browning BTC 5HD, Browning, Morgan, UT) at each camera trap site during three sampling sessions in 2016 and 2017 to estimate mesocarnivore occupancy among grazing systems. Mesocarnivores typically prefer to hunt and forage along habitat edges (Andr n 1995). We used ArcMap 10.4 to digitize the habitat edges that may influence mesocarnivore occupancy, such as wooded draws, fencelines, roads, and water features (ESRI 2011, v10.4).

We conducted all analyses using the statistical program R (R Core Team, 2016), with

use of packages ‘unmarked’ (Fiske and Chandler, 2011) and ‘AICcmodavg’ (Mazerolle, 2013) to identify specific effects on occupancy of mesocarnivores. Occupancy modeling allowed for simultaneous estimation of detection probability and occupancy (MacKenzie et al., 2006).

RESULTS AND DISCUSSION

We recorded 178 mesocarnivore detections during 3,535 camera trap days at 164 remote camera sites, which were located within 8 pastures managed for cattle grazing. Sixteen of the original 180 camera sites were censored from the analysis due to lack of spatial independence (12), camera malfunction (2), cattle destruction (1), or camera theft (1). We detected 5 species of mesocarnivores during camera trap surveys: American badger, coyote, raccoon, striped skunk, and long-tailed weasel. We pooled observations for analysis, as previous researchers have suggested, in grassland ecosystems with relatively high predator diversity, management actions to reduce grassland bird nest depredation may not prove successful if they do not consider the entire predator community (Renfrew and Ribic 2003, Pietz et al. 2012). The mean (\pm SE) probability of detecting a mesocarnivore at the study area was 0.3 ± 0.03 . Five models shared support (Δ AICc ≤ 2.0) for management-level effects on mesocarnivore occupancy. The top model included the main effects of grazing system and stocking rate ($w_i = 0.24$). Mesocarnivore occupancy was highest in rest-rotation grazing systems, followed by season-long ($\beta = -3.54 \pm 2.49$) and summer-rotation systems ($\beta = -3.44 \pm 2.10$), and the probability of mesocarnivore occupancy increased with stocking rate ($\beta = 1.64 \pm 1.10$; Figure 1). The probability of mesocarnivore occupancy decreased sharply with distance to nearest fence ($\beta = -1.92 \pm 0.64$).

Low livestock stocking rates typically result in higher cattle forage selectivity and higher within-pasture variation in vegetation structure, when compared to high stocking rates (Fuhlendorf and Engle, 2001; Toombs et al., 2010), which may act to decrease predator search efficiency (Bowman and Harris, 1980). We

found mesocarnivore occupancy increased with stocking rate, and these results were consistent with previous research suggesting that livestock grazing may facilitate increased use by mesocarnivores (Gese and Thompson, 2014). Within contiguous rangeland landscapes such as our study area, we hypothesized that fences may represent effective habitat edges, potentially due to their physical structure or differences in vegetation structure among pastures. We found a negative relationship between mesocarnivore occupancy and distance to the nearest fence, which was consistent with previous work regarding mesocarnivore use of fencelines (Pedlar et al., 1997; Smith et al., 2017). In areas beyond 500 m from fences, predicted mesocarnivore site occupancy declined sharply.

Our results have implications for mesocarnivore prey species occupying rangelands managed with multi-pasture rotational livestock grazing systems, such as rest-rotation or short-duration grazing systems. Intensively managed grazing systems often use fencing to divide larger pastures into multiple

smaller pastures, to achieve the desired forage utilization as determined by the range manager (Hart et al., 1993). However, adding more fence to the landscape may facilitate mesocarnivore occupancy or improved foraging efficiency in these areas, potentially reducing survival or reproductive output of grassland birds and small mammals, common mesocarnivore prey in grassland ecosystems.

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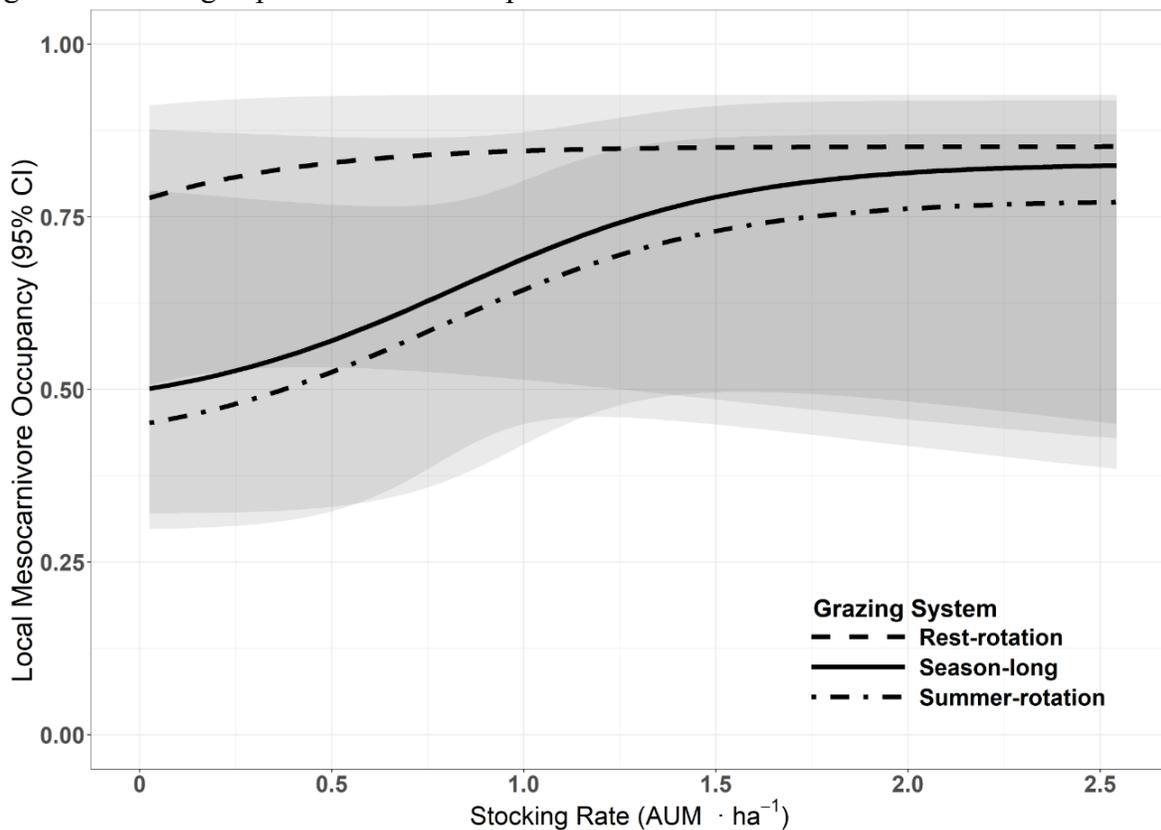


Figure 1. The top management-level model describing local occupancy of mesopredators in eastern Montana during 2016–17 included main effects of grazing system and stocking rate.

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*Corresponding author email:
voldskyler@gmail.com