Beef Cattle Sciences

Oregon Beef Council Report

2017 Edition

Oregon State University

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Thank you for the interest in the 2017 - Oregon Beef Council Report. This publication contains information about research studies funded by the Oregon Beef Council, and conducted by faculty members from Oregon State University. For questions, suggestions, or comments regarding this publication, please contact Rodrigo Marques (541-573-4083 or marquesr@oregonstate.edu) or David Bohnert (541-573-8910 or dave.bohnert@oregonstate.edu)

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Increasing milk production in bovine mammary cells: a nutrigenomic strategy [1]

Massimo Bionaz[2] and Jayant Lohakare[3]

Synopsis

Use of a compound that inhibits a specific nuclear receptor previously demonstrated to negatively affect glucose transport failed to increase synthesis of lactose in bovine mammary cells.

Summary

The long term goal is to put tools in the hand of producers to aid in improving the performance and health of animals by modifying the animal's biology via nutrient-gene interaction. The aim of this study was to evaluate the possibility of increasing milk lactose production by nutrigenomic intervention. The objective of this experiment was to evaluate the effects of modulating the nuclear receptor peroxisome proliferator-activated receptor beta/delta (PPARβ/δ) on glucose import and lactose synthesis in bovine mammary epithelial cells. For this purpose we performed 3 subsequent experiments using immortalized and primary bovine mammary cells. In Experiment 1 we determined the dose of two compounds that maximize the inhibition of the PPARβ/δ in immortalized cells. Once we determined the dose of the inhibitor we performed Experiment 2, where we tested if the modulation of PPARβ/δ increase the synthesis of lactose in cells cultivated on plastic. To fully recapitulate the production of milk-related compounds in mammary cells we have performed Experiment 3, where we cultivated the cells in collagen, aiding the formation of a 3-dimensional structure in the attempt to increase the production of milk components, including lactose. We measured glucose, lactose, and expression of key genes in the metabolism of glucose and lactose synthesis.

Overall, the data indicated no effect of the modulation of PPARβ/δ on the uptake of glucose or synthesis of lactose in bovine mammary cells.

Introduction

Lactose content in bovine milk is approximately 4 to 5% (Davies et al., 1983). Milk yield greatly depends on mammary lactose synthesis due to its osmolality property that induces mammary uptake of water. Therefore, the rate of lactose synthesis in the epithelial cells of the mammary gland serves as a major factor influencing milk volume. The supply of glucose for lactose synthesis increases dramatically in the mammary gland of lactating animals. It has been estimated that mammary tissue extracts 60-85% of glucose from blood (Annison and Linzell, 1964, Rigout et al., 2002). Being glucose the main precursor for lactose synthesis, the uptake of glucose

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by the mammary gland can play a major role in regulating the final milk volume. Therefore, modulating the glucose uptake by the mammary gland ultimately should improve dairy productivity and efficiency of milk production.

The nuclear receptor Peroxisome Proliferator-activated Receptor beta/delta (PPARβ/δ) controls expression of several genes involved in lipid catabolism; however, in bovine its activation also inhibits glucose uptake. This was demonstrated in bovine aortic endothelial cells where activation of PPARβ/δ by peroxides, produced *via* 12-lipoxygenase enzyme as a consequence of high-glucose, decreased the amount of mRNA coding for the solute carrier family 2 member 1 (*SLC2A1*) also called GLUT1 (Riahi et al., 2010), among the most important glucose transporter in bovine mammary tissue (Zhao and Keating, 2007, Bionaz et al., 2012a). The decreased mRNA of *SLC2A1* was a result of an increase in expression of calreticulin *via* activation of PPARβ/δ. The calreticulin is a protein that increases degradation of GLUT1 mRNA. Therefore, the inhibition of glucose uptake was due to an increased degradation of GLUT1 mRNA by calreticulin.

The expression of PPARβ/δ gene is very abundant and is significantly down-regulated from pregnancy to lactation in bovine mammary tissue (Bionaz et al., 2012b, Bionaz et al., 2013) concomitantly with an increase in expression of several glucose transporters including *SLC2A1* (Bionaz and Loor, 2011, Bionaz et al., 2012b).

In lieu of above, it appears that any natural or synthetic compound that can inhibit PPARβ/δ, thus, augmenting the expression of glucose transporters in mammary gland and decreasing utilization of glucose *via* glycolysis, has prospects to increase amount of glucose channeled toward lactose synthesis, thereby increasing milk production in the mammary gland.

Based on the above presented data we hypothesize that PPARβ/δ plays a major role in modulating glucose uptake in bovine mammary epithelial cells affecting synthesis of lactose (Figure 1). In order to test the hypothesis we used immortalized bovine mammary alveolar cells (MACT) and primary mammary bovine cells to test: 1) the most effective dose to inhibit PPARβ/δ activity among two synthetic antagonists; 2) to assess the effect of PPARβ/δ activation and inhibition on glucose uptake and lactose synthesis by measuring glucose, lactose, and expression of related key genes in cells cultured in plastic dishes; and 3) to test the effect of PPARβ/δ activation and inhibition on glucose uptake and lactose synthesis by measuring glucose and lactose in cell cultured in Matrigel.

Materials and Methods

Cell Culture, Transfection and Treatments

Mammary alveolar cells transformed (MACT) and primary bovine mammary epithelial cells (PBMC) isolated from another laboratory (Hu et al., 2009) were used for the *in vitro* studies. Culture medium was changed every 48 h and cells were subcultured to 70 to 80% confluence (approximately every 3 to 4 d).

Experiment 1. Approximately, 25,000 cells/well were plated in 96-well plate. Twenty four hours later the cells were co-transfected with a PPAR Response Element associated with llucifgerase and a renilla plasmid at 50:1 ratio of luciferase/renilla plasmid. After 24 h of transfection, the cells were treated either with PPARβ/δ antagonists GSK-3787 and PT-S58 in quadruplicates at 10, 100, 1000 and 10000 nM with the PPARβ/δ agonist GW501516 at 1000 nM, with ethanol (as control) or only media in high-glucose DMEM media with or without FBS. Luciferase and renilla activity were measured *via* luminometer. The experiment was also run using a robotic Digital Dispenser.

Experiment 2: To study the effects of PPARβ/δ activation and inhibition on glucose uptake and lactose synthesis and expression of related genes, approximately 20,000 MACT and PBMC cells were plated in 24-well plates. Based on the results of Experiment 1, the cells were treated in quadruplicates with GW501516 at 1000 nM, GSK3787 at 1000 nM and their combination (GW501516 + GSK3787 at 1000+1000 nM), and ethanol as control. After 24 h treatment, the media

was collected and stored at -20ºC. The number of live cells were counted and RNA extracted to measure expression of glucose transporter 1 (*SLC2A1*), calreticulin (*CALR*), alpha-lactalbumin (*LALBA)*, hexokinase 1 (*HK1*), pyruvate dehydrogenase kinase, isozyme 4 (*PDK-4*), and peroxisome proliferator-activated receptor delta (*PPARD*) using RT-qPCR technique.

Experiment 3. Approximately 20,000 cells were cultivated in BioCoat™ Matrigel® in order to induce alveolar-like formation (i.e., tridimensional structure) that allows for expression of milk-related proteins (Bionaz et al., 2012a). Cell were treated as for Experiment 2. Glucose and lactose were measured in the media using assay Kits

Statistical Analysis

Prior to statistical analysis data were checked for outliers using PROC REG of SAS 9.3 (SAS Institute, Inc., Cary, NC, USA). The GLM procedure of SAS was used to evaluate the treatments effect for all the parameters measured. Fixed effects in the model were treatments, whereas the random effects were replicates (n = 4 replicates/treatment). Significance was declared at $P \leq 0.05$.

Results

Experiment 1. Significant activation of PPARβ/δ was obtained by GW501516 as expected (Oliver et al., 2001). The most effective inhibition of PPARβ/δ was obtained by ≥1,000 nM of GSK-3787, which was consistent and independent of the presence of FBS (Figure 2). The PT-s58 failed to consistently inhibit PPARβ/δ (Figure 2). In the absence of FBS and in the presence of ethanol (but not DMSO) all the doses of PT-s58 and 100 nM GK-3787 activated PPAR isotypes (Figure 1A). The use of the digital dispenser with DMSO as eluent provided a more consistent response compared to the use of manual method with ethanol as eluent (Figure 1). The reason for such an effect is unclear.

Experiment 2. Figure 3 depicts the effect of the treatments on the transcript abundance of genes related to glucose metabolism. Activation of PPARβ/δ by GW501516 in MACT and PBMC increased the transcript abundance of *PDK4*, a known PPARβ/δ target gene (Ordelheide et al., 2011) and one of the main regulators of the use of glucose for fatty acid synthesis (Kwon and Harris, 2004). Contrary to what previously reported (Riahi et al., 2010), the same treatment decreased the transcription of *CALR* but only in MACT cells with no effect on PMBC. In MACT cells PPARβ/δ agonist and antagonist, but not the combination of them, decreased the transcript abundance of *PPARD* while the abundance of the same transcript was induced by FBS with only a numerical additional increase by the PPARβ/δ agonist and antagonist. None of the treatments had a significant effect on the transcript abundance of *SLC2A1*, coding for one of the main glucose transporters in bovine mammary cells (Zhao and Keating, 2007, Bionaz and Loor, 2011), and the *HK1*, coding for the enzyme responsible for the activation of glucose before being used in most of the glucose-utilization pathways. The transcript abundance of GLUT8 (*SLCA8*), another important glucose transporter in bovine mammary (Zhao and Keating, 2007, Bionaz and Loor, 2011), was significantly downregulated by the activation of PPARβ/δ but only in MACT cells while in PBMC the abundance of the same transcript was induced by inhibition of PPARβ/δ; however, in both cases the effect was significant compared to ethanol control but was not significantly different compared to the other treatments.

The transcript of *LALBA*, coding for the main enzyme involved in lactose synthesis (Osorio et al., 2016), was below the limit of detection (i.e., Cq >32) in PMBC. In MACT cells abundance of *LALBA* was numerically higher compared to the ethanol control using the agonist and the antagonist of PPARβ/δ. For the PMBC we also used *GAPDH* as a target gene but was not affected significantly by PPARβ/δ modulation.

Overall, data of the present work indicated that the dose of GSK3787 that inhibited the transactivation of PPARβ/δ (see Experiment 1) failed to consistently affect the expression of PPARβ/δ target gene *PDK4*. Our data failed to recapitulate what previously observe in bovine endothelial cells, especially the fact that *CALR* is a

PPARβ/δ target in bovine (Riahi et al., 2010). This is a central piece of the hypothesis of the present paper (Figure 1). Furthermore, we detected difference in the response of the two cell types to the same treatments.

Figure 4 (upper panels) depicts the effect of the treatments on the number of cells, glucose uptake, lactose production, and the proportion of glucose used for lactose production in bovine mammary cells cultivated in plastic. As for the gene expression, we detected a different response of the type of bovine mammary cells to the treatments. In MACT cells the inhibition of PPARβ/δ did not significantly affected the glucose uptake, lactose produced, or the number of cells. The same treatment however had a larger amount of glucose used for lactose synthesis compared to the activation of PPARβ/δ (Figure 4). The same effect was not recapitulated in PMBC, where we actually detected an overall lower amount of cells with the use of PPARβ/δ agonist. The same treatment increased the cellular uptake of glucose and the cellular production of lactose without affecting the amount of glucose used for lactose synthesis.

Experiment 3. PMBC cultivated in Matrigel tended to form tridimensional structures but they failed to produce clear alveolar-like structure as expected (Jedrzejczak and Szatkowska, 2014). We did not observe any tridimensional structure formation by MACT. Despites this, compared to cultivation in plastic, both cell types had a significant larger uptake of glucose, larger secretion of lactose, and greater utilization of glucose to produce lactose when cultivated in Matrigel vs. plastic. However, the modulation of PPARβ/δ showed no effects on cell numbers, estimated glucose uptake/cell and lactose synthesis/cell in MACT cells grown Matrigel, while we detected a larger lactose production/cell (driven by a numerical larger glucose uptake) when PPARβ/δ was activated in PBMC (Figure 4). The effects observed in cells cultivated in plastic were not recapitulated when cells were cultivated in Matrigel.

Conclusions

Based on the results it is concluded that the data do not provide a strong support for PPARβ/δ being involved in glucose uptake and lactose synthesis

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Acknowledgments

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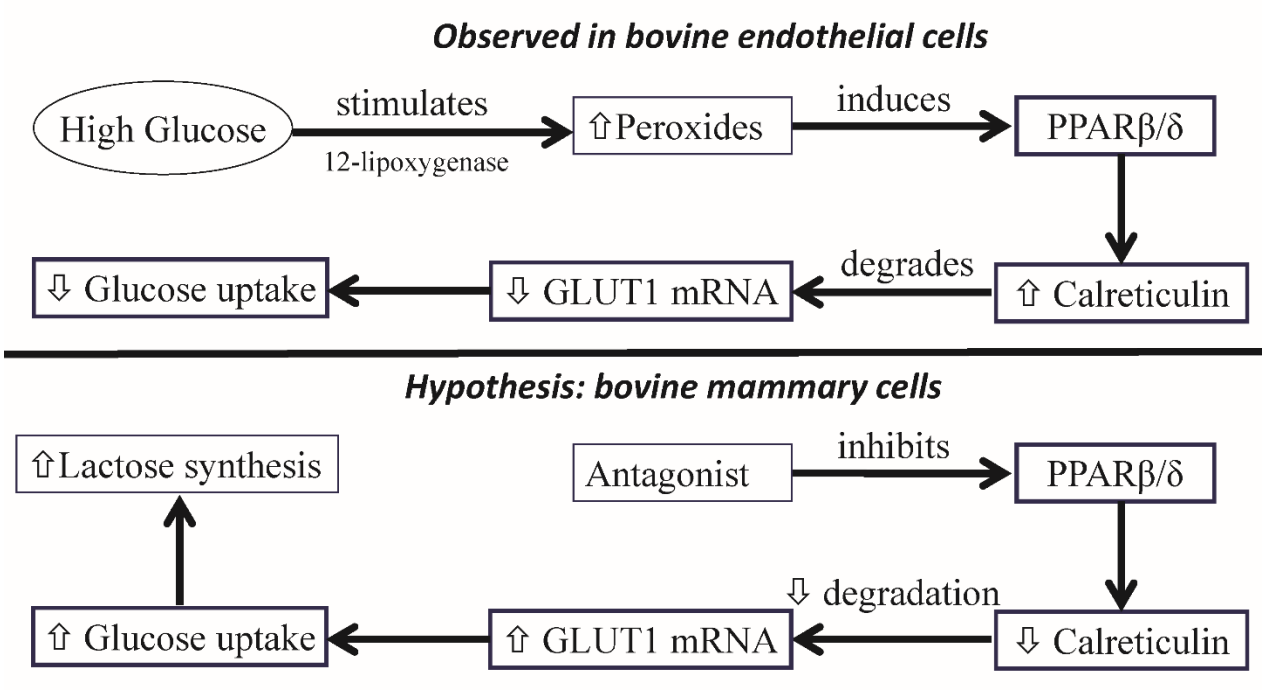

Figure 1. Schema of previous data obtained in endothelial bovine cells which were the foundation for the hypothesis of the present paper

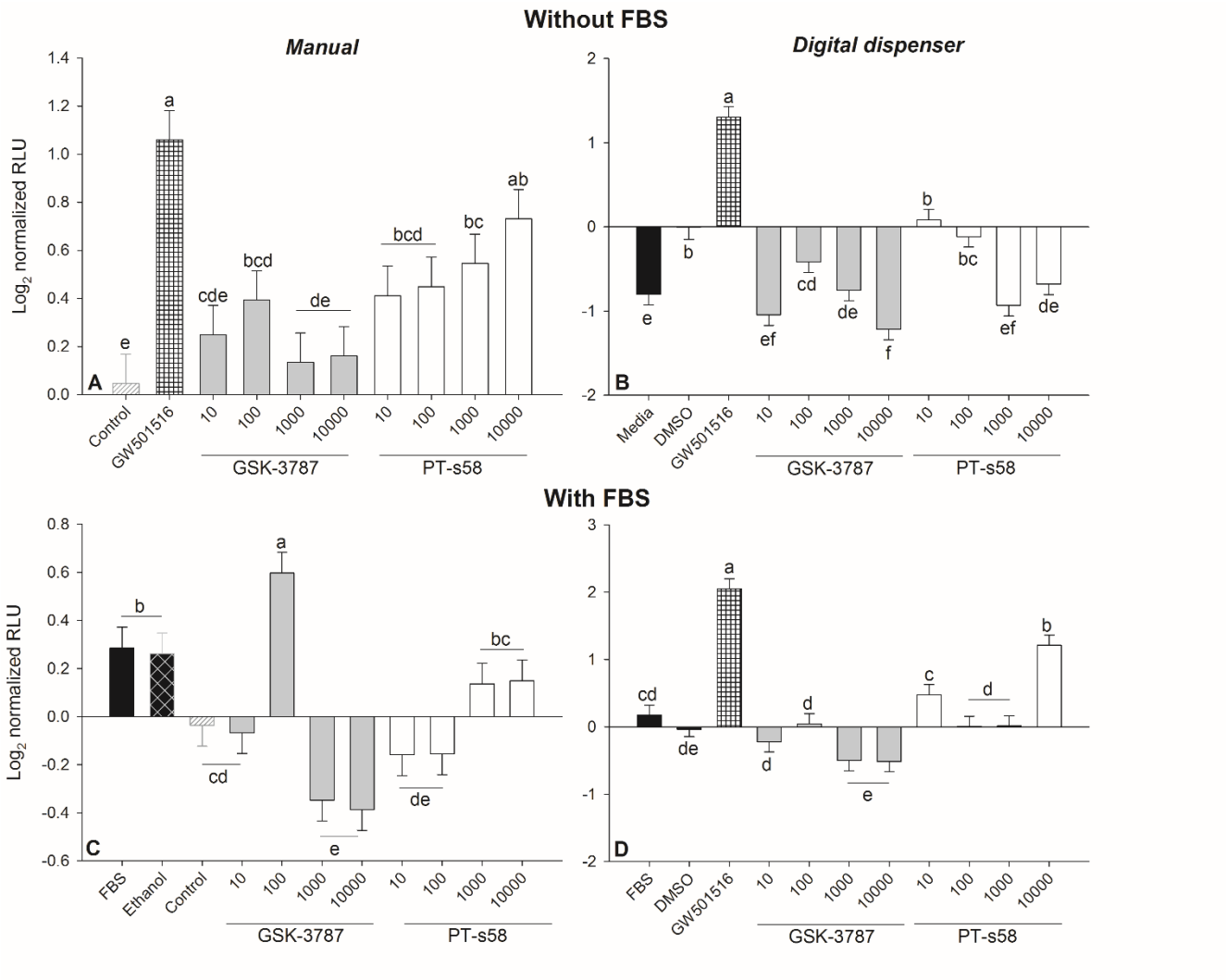

Figure 2. Figures A and C depict the results of manual addition of treatments. Figures B and D depicts the results of addition of treatments using the HP D300e Digital Dispenser. Compound and doses are reported on X-axis. Different letter denote a significance with P-value<0.05.

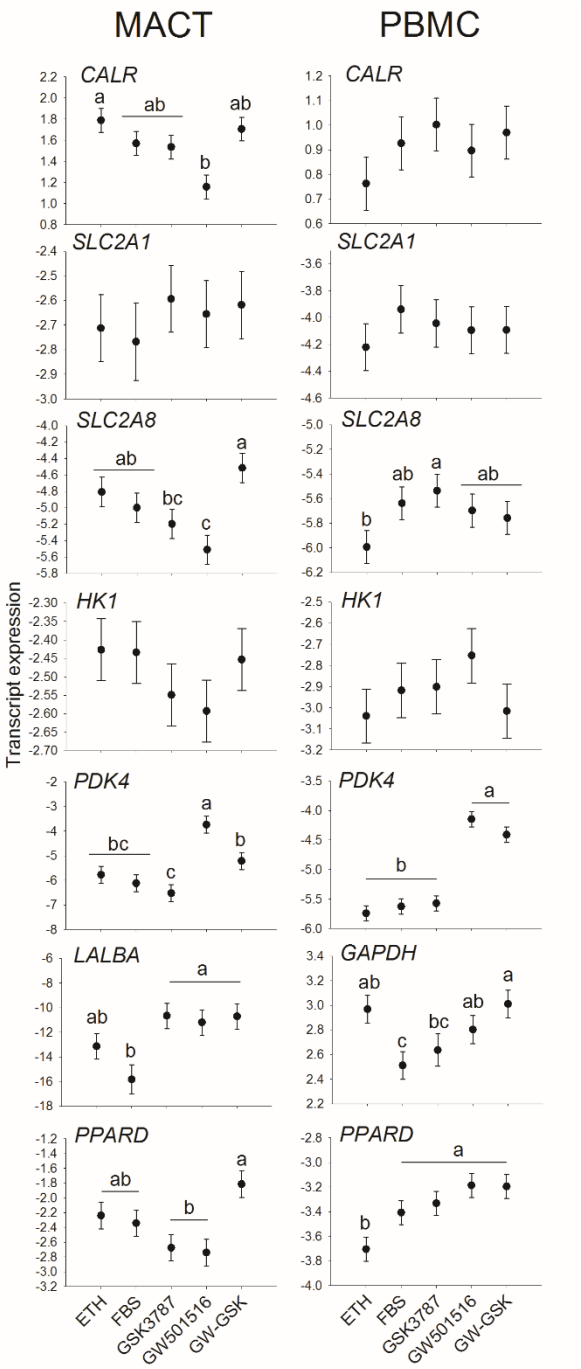

Figure 3. Expression of various genes involved in glucose metabolism and lactose synthesis in bovine mammary epithelial cells. In X-axis are reported the various treatments and in Y-axis the relative expression. Different letters denote significance with P-value<0.05.

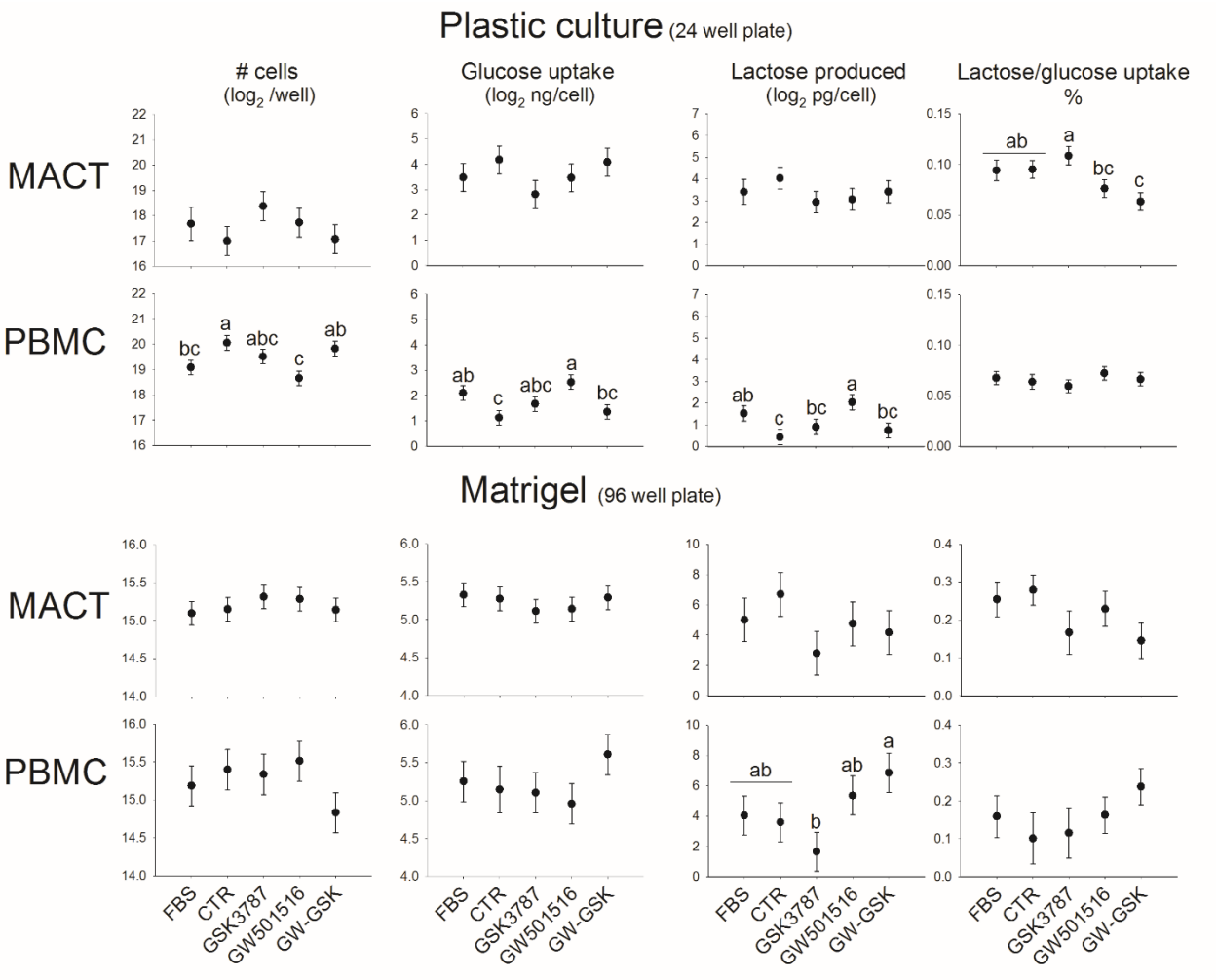

Figure 4. Uptake of glucose, number of cells, lactose synthesis and amount of glucose used for lactose synthesis in bovine mammary cells treated with activator and inhibitor of PPARβ/δ. Different letter denote significance with P-value<0.05.

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Feeding immunostimulant ingredients to optimize health and performance of receiving cattle [1]

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Synopsis

Research is still required to identify nutritional strategies that enhance performance, health and physiological variables of high-stress receiving cattle

Summary

One hundred and eight Angus × Hereford steers, originating from 7 cow-calf ranches and weaned on d -3, were obtained from an auction yard on d -2 and road-transported (500 miles; 12-h) to an experimental feedlot facility. Upon arrival on d -1, shrunk weight was recorded and steers were grouped with free-choice access to grass hay, mineral supplement, and water. On d 0, steers were ranked by source and shrunk weight, and assigned to 1 of 18 pens (6 steers/pen). Pens were allocated to: 1) no *immunomodulatory ingredient* supplementation (CON), 2) supplementation with Omnigen-AF (OMN; 22 g/steer daily, as-fed basis; Phibro Animal Health, Teaneck, NJ) from d 0 to 30, or 3) 2 oral capsules of Stocker Immune Primer on d 0 + 15 g/steer daily (as-fed basis) of Stocker Preconditioned Premix (Ramaekers Nutrition; Santa Cruz, CA) from d 7 to 30 (IPF). From d 0 to 80, steers had free-choice access to grass hay, water, and received a corn-based concentrate. Feed intake was recorded from each pen and steers assessed for bovine respiratory disease (BRD) signs daily. Steers were vaccinated against BRD pathogens on d 0 and 21. Final shrunk weight was recorded on d 81, and blood samples were collected on d 0, 3, 7, 10, 14, 21, 31, 42, 56, and 73. Steer average daily gain and final weight were greater ($P \leq 0.05$) in CON vs. OMN and IPF (2.71, 1.67, and 2.35 lbs/d; 704, 621, and 677 lbs; respectively), and ($P < 0.01$) in IPF vs. OMN. No treatment effects were detected ($P \geq 0.76$) for BRD incidence (66 ± 4%) and feed intake, whereas feed efficiency was greater ($P < 0.01$) in OMN vs. CON. Plasma haptoglobin concentrations tended ($P = 0.10$) to be greater in CON vs. IPF on d 3, were greater ($P = 0.04$) in IPF vs. CON on d 7, and tended ($P = 0.10$) to be less in OMN vs. IPF and CON on d 21. Collectively, the *immunomodulatory feed* ingredients evaluated herein impacted innate immune responses, but failed to mitigate BRD incidence and improve performance of receiving cattle.

Introduction

Feedlot receiving is one of the most critical phases within the beef production cycle, when cattle are exposed to a multitude of stress and health challenges that directly impact animal welfare and productivity throughout the feeding period (Cooke, 2017). Receiving cattle not only already experienced road transport, but are immediately subjected to stress caused by commingling with different animals and exposure to novel diets and environments, which are all known to directly impair their immune system. Accordingly, incidence of bovine respiratory

1. This document is part of the Oregon State University – 2017 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.\
2. Oregon State University – Eastern Oregon Agricultural Research Center, Burns.

diseases (**BRD**) is extremely elevated during the initial 30 days of feedlot receiving, with clinical symptoms observed in up to 60% of receiving cattle (U.S. average) despite efforts associated with stress minimization and vaccination against BRD pathogens (Kirkpatrick et al., 2008).

The BRD complex is the most costly disease of feedlot cattle in the US, and costs the national beef industry approximately $ 1 billion annually. These economic losses include, besides cattle mortality, costs associated with reduced performance of morbid cattle and purchase of antibiotics (Loerch and Fluharty, 1999). With the increased regulations and pubic/consumer concern regarding antibiotic use in beef production systems, strategies that naturally enhance cattle immune function and boost vaccine efficacy are warranted to reduce BRD incidence and optimize productivity in feedlot cattle. Such strategies include the use of immunostimulant feed ingredients into receiving diets, which are based on nutritional and scientifically-sound compounds, but that still require proper validation in feeder cattle. One example is Omnigen-AF® (Prince Agri Products, Quincy, IL) a feed ingredient based on yeast and vitamins that has been shown to optimize health and milk production of early-lactating dairy cows. Another example are the Livestock Stress Formulas from Ramaekers Nutrition (Santa Cruz, CA), which are based on bioactive peptides and oligosaccharides that are naturally-occurring in colostrum, and that have been used by commercial feedyards with encouraging field results. Therefore, research is warranted to fully evaluate the potential of these ingredients to optimize health and productivity of receiving cattle, with thorough evaluation of immune parameters, vaccine efficacy, BRD incidence, and performance traits during feedlot receiving.

Hence, this experiment evaluated the impacts of immunostimulant ingredients (Omnigen-AF® or Livestock Stress Formulas) on performance, health and physiological variables of receiving cattle

Materials and Methods

This experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (EOARC; Union, OR). Animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee.

One hundred and eight recently-weaned Angus x Hereford steers were purchased from a commercial auction yard (Producers Livestock Marketing Association: Vale, OR). Steers were originated from 7 cow-calf operations located in Eastern and Central Oregon. On the day of purchase (day -2 of the experiment, 9/14/2016), steers were loaded into a commercial livestock trailer and transported for 500 miles (12 h) to stimulate the stress of a long-haul. On day -1 of the experiment, steers were unloaded at the EOARC Union, tagged, arrival shrunk body weight was recorded, and calves were maintained as a single group with free-choice hay, water, and mineral supplement for 24 h.

On day 0 of the experiment, steers were ranked according to source and body weight, and allocated to an 18-pen drylot (6 steers/pen, with steers from at least 3 different sources within pen). Pens were randomly assigned to receive 1 of 3 treatments:

- Control: No immunostimulant supplementation (**CON**).
- Omnigen-AF®: Supplementation with Omnigen-AF® (22 g/steer daily; Prince Agri Products, Quincy, IL) from day 0 to 30 of the experiment (**OMN**).
- Livestock Stress Formulas: Two capsules of Stocker Immune Primer Release with trace minerals (Ramaekers Nutrition; Santa Cruz, CA) on day 0, in addition to 15 g/steer daily of the Stocker Preconditioned Premix (Ramaekers Nutrition) from day 7 to 30 of the experiment (**LSF**).

On day 0, steers were vaccinated against Clostridium (One Shot Ultra 7; Zoetis, Florham Park, NJ), parainfluenza virus, infectious bovine rhinotracheitis virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (Bovi-Shield Gold One Shot; Zoetis), and administered an anthelmintic (Dectomax; Zoetis). On day 21, steers were re-vaccinated against bovine rhinotracheitis virus, bovine viral diarrhea Types 1 and 2 viruses, parainfluenza3 virus and bovine respiratory syncytial virus (Bovi-Shield Gold 5; Zoetis), and *Clostridium* (One Shot Ultra 7; Zoetis).

Steers received a free-choice receiving diet based on mixed alfalfa-grass hay, cracked corn, and soybean meal with a forage:concentrate ratio of 75:25 from day 0 to 7, 60:40 from day 8 to 18, and 40:60 from day 18 to day 30, and 30:70 from day 31 to 80. Feed intake was recorded daily by measuring offer and refusals from each pen.

Steer body weight and blood samples were collected on days 0, 3, 7, 10, 14, 21, 31, 42, 56, 70, and 80 of the experiment. Steer shrunk body weight was also collected on day 81 for average daily gain calculation, using shrunk weight on day -1 as initial weight. Blood samples were analyzed for plasma haptoglobin concentrations.

Steers were observed daily for bovine respiratory disease (**BRD**) symptoms, and treated with an antimicrobial when clinical symptoms are observed. In addition, steers from the LSF group received 2 capsules of Stocker Immune Primer Release with trace minerals concurrently with each antimicrobial administration. Incidence of respiratory treatments, morbidity, and mortality were recorded daily

Statistical analysis

Pen was considered the experimental unit. All data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc.; version 9.3) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10.

Results

As designed, initial body weight (day -1) was similar ($P = 0.99$) among treatments (Table 1). However, average daily gain during the 80-day receiving period was greater ($P \leq 0.05$) in CON cattle compared with LSF and OMN cattle, and also greater for LSF vs. OMN cattle (Table 1). Consequently, CON cattle were the heaviest ($P \leq 0.05$), followed by LSF cattle, and OMN cattle were lighter at the end of the receiving period (Table 1). It is important to note, however, that body weight was similar ($P \geq 0.85$) among all treatment groups until day 56 of the experiment, but growth rates differed significantly ($P \leq 0.05$) among all treatment groups from day 56 to the end of the receiving period (Figure 1).

No differences in feed intake were detected ($P \geq 0.77$) between treatments during the experiment. It is also important to note that feed intake remained similar ($P \geq 0.94$) between treatments after day 56 (Figure 2). Therefore, treatment differences detected on body weight after day 56 should not be associated with a drop in feed intake, but with reduced ($P \leq 0.05$) feed efficiency during this period (Table 1).

Accordingly, feed efficiency during the entire 80-day feeding period was less ($P < 0.01$) in OMN vs. CON cattle (Table 1). On the other hand, the decreased average daily gain of LSF cattle was not sufficient to negatively impact their overall feed efficiency calculation, which was similar ($P = 0.30$) compared with CON cattle and greater ($P = 0.03$) compared with OMN cattle (Table 2).

No treatment differences were detected ($P \geq 0.55$) for health parameters, including incidence of BRD symptoms (Figure 2), number of antimicrobial treatments required per sick animal, as well as % of cattle that needed to be treated with antimicrobials more than once (Table 2). No treatment differences were also detected ($P = 0.36$) for mortality during the experiment (Table 2). Note that incidence of BRD was substantial during this experiment, particularly during the initial 28-days of receiving. Plasma concentrations of haptoglobin did not differ ($P = 0.74$) among treatments (Figure 4), but peaked on day 3 of the experiment denoting the substantial physiological stress and inflammation that steers were exposed to upon feedlot entry.

Conclusions

Hence, this experimental model fully represented the stress and health challenges that commercial feeder cattle experience during feedlot receiving, resulting in elevated BRD incidence and morbidity. However, none of the treatments evaluated herein were capable of mitigating these challenges, and actually reduced overall 80-day receiving performance compared with steers not receiving any immunomodulatory supplement. Hence, research is still required to identify nutritional strategies that enhance performance, health and physiological variables of high-stress receiving cattle.

Acknowledgements

This research study was financially supported by the Oregon Beef Council.

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Table 1. Growth and intake parameters during the initial 80-day receiving period. Values with different superscripts are statistically different ($P \leq 0.05$)

Item	CON	LSF	OMN	SEM	*P*-value
Initial body weight (day -1), lbs	482	484	484	17	0.99
Final body weight (day 80), lbs	704[a]	677[b]	621[c]	10	< 0.01
Average daily gain (day 0 to 80), lbs/day	2.71[a]	2.35[b]	1.67[c]	0.13	< 0.01
Feed intake, lbs of dry matter/day					
Hay	7.01	6.74	6.82	0.55	0.93
Concentrate	10.21	10.18	10.32	0.13	0.77
Total	17.23	16.92	17.14	0.65	0.94
Feed efficiency (lbs gain/lbs feed intake)					
Overall (day -1 to 80)	0.173[a]	0.152[a]	0.107[b]	0.014	< 0.01
Day 56 to 80	0.229[a]	0.176[b]	0.050[c]	0.027	< 0.01

Table 2. Health parameters during the initial 56 days of the experiment.

Item	CON	LSF	OMN	SEM	*P*-value
Incidence of BRD symptoms, %	69.4	61.1	69.4	9.1	0.76
Treated calves that required re-pull, %	13.1	23.3	27.3	9.9	0.59
Number of treatments required	1.13	1.32	1.31	0.13	0.55
Mortality, %	2.8	5.5	0.0	2.7	0.36

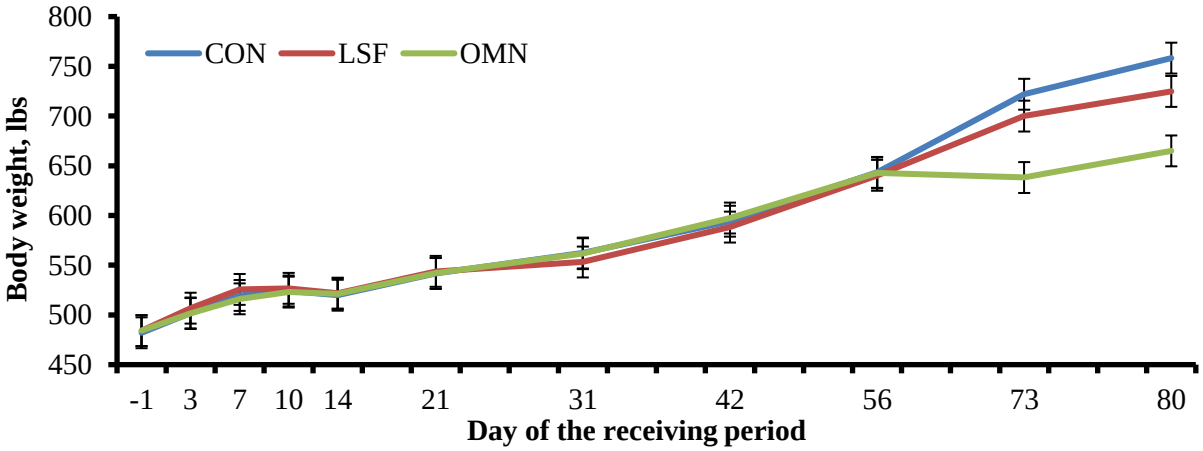

Figure 1. Growth rate during the 80-day receiving period. From day 56 to 80, body weight gain was less ($P \leq 0.05$) in LSF and OMN compared with CON steers, and also less ($P \leq 0.05$) in OMN compared with LSF steers.

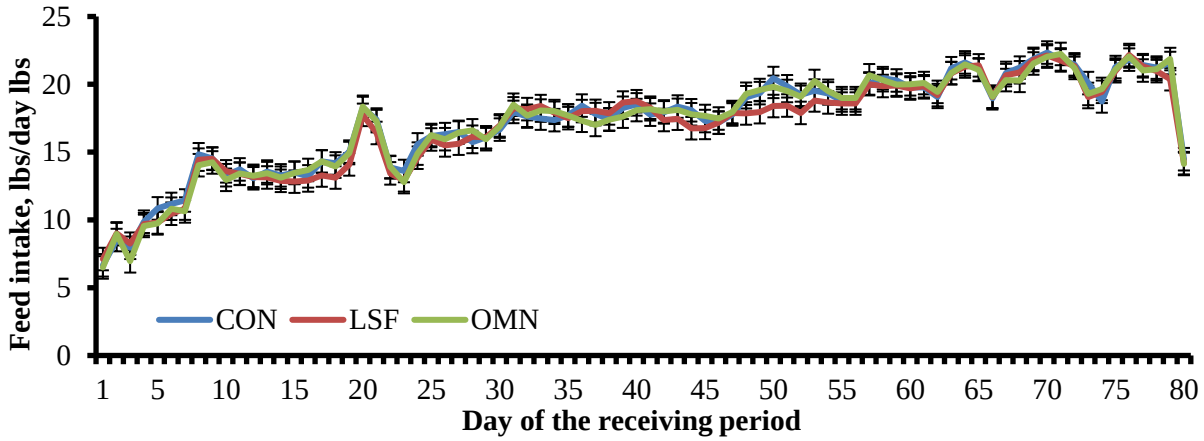

Figure 2. Feed intake (hay + concentrate) during the 80-day receiving period. No treatment differences were detected (P = 0.94).

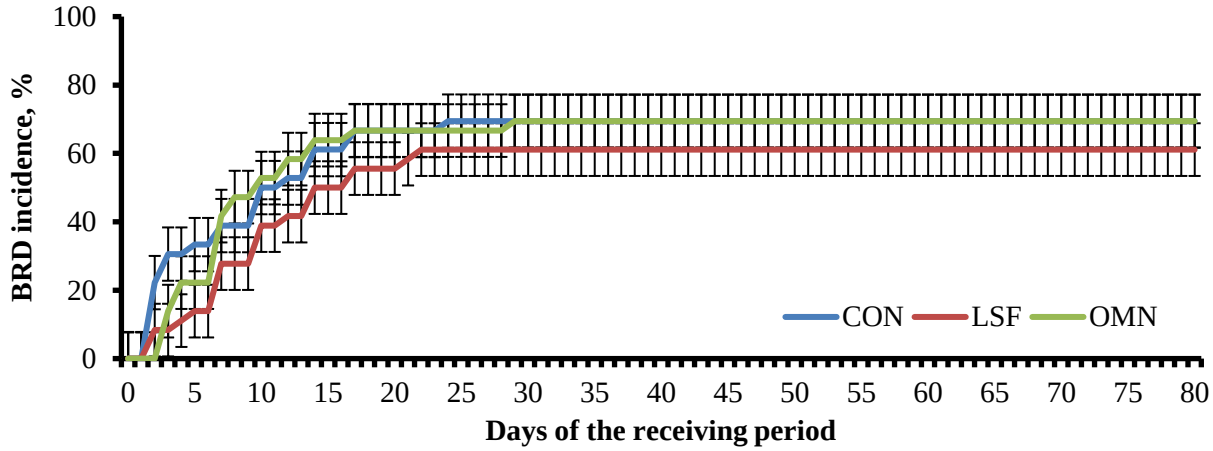

Figure 3. Cumulative incidence of BRD symptoms that required antimicrobial treatment during the experiment. No treatment differences were detected (P = 0.59).

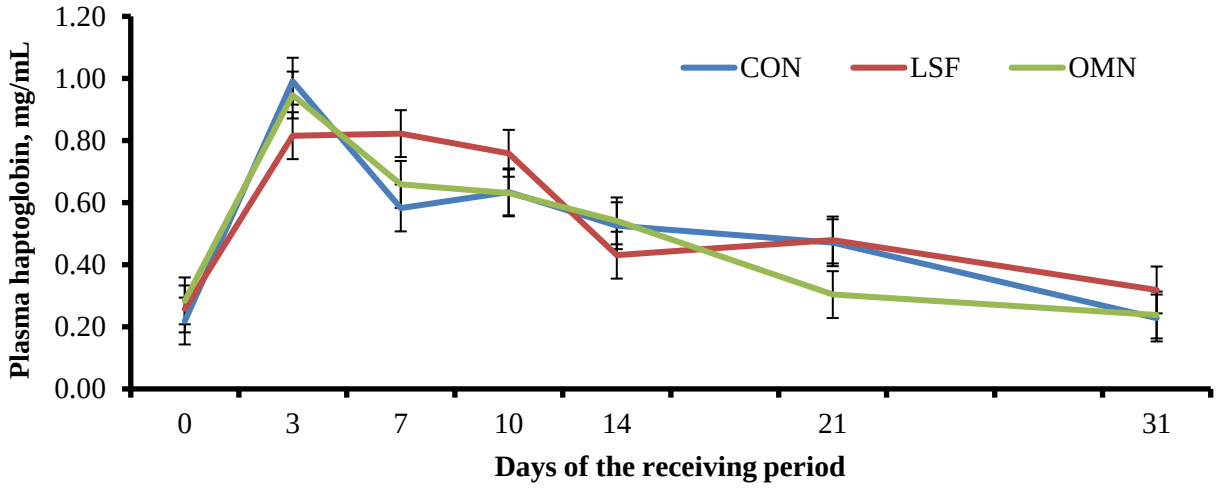

Figure 4. Plasma haptoglobin concentrations during the first 31-days of the experiment. No treatment differences were detected (P = 0.74).

BEEF154

Oregon Beef Council Report

Beef Cattle Sciences

Development of an enhanced cattle embryo transfer medium to improve pregnancy rates in embryo transfer recipients [1]

Alexandria Snider[2], Nicole Steigerwald[3] and Alfred R. Menino, Jr.[4]

Synopsis

Recipient conception rates improve when cows receive embryos strawed in a newly designed, synthetic enhanced transfer medium (ETM). The new transfer medium can be easily merged into current procedures and would be an applicable strategy for improving conception rates in embryo transfers.

Summary

The specific aim of this research was to evaluate strategies for improving conception rates in embryo transfer recipients that would be compatible with transfer procedures presently used by the industry. The average conception rate for cows used as recipients in nonsurgical transfers with fresh embryos is 61% so there is room to improve this percentage. The period between collecting and transferring the embryos offers a window of time where embryos could be incubated briefly (< 2 h) in a culture medium that enhances or stimulates their development prior to transfer. Alternatively, embryos could be strawed in a similar medium thereby transferring both embryo and modified medium to the recipient's uterus. The first experiment was a "proof of concept" undertaking where embryos were incubated immediately after collection in a modified culture medium containing 0 or 100 µg/ml plasmin for 16 h. Although the difference was not significant because the number of transfers was low (n = 48), the conception rate was 12% greater in recipients receiving embryos incubated for 16 h in medium containing 100 vs. 0 µg/ml plasmin. Incubating embryos for 16 h would be difficult to integrate into current transfer procedures, so in the second experiment conception rates were evaluated in recipients receiving embryos incubated for 2 h in medium containing a higher dose of plasmin, 200 vs. 0 µg/ml. The higher plasmin dose for the shorter time had no effect on conception rate and, in fact, conception rates were identical (52%). The third experiment compared conception rates in embryos strawed in either the conventional transfer medium or a new, modified embryo transfer medium containing 200 µg/ml plasminogen. Conception rates were greater in recipients receiving embryos strawed in the new plasminogen-containing medium (52%) compared to the conventional medium (38%), however the difference was not statistically significant. As the strategy where strawing embryos in the new medium seems the most applicable for the cattle industry,

1. This document is part of the Oregon State University – 2017 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.\
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more embryo transfers are planned for the coming year.

Introduction

A popular applied reproductive technology used to improve herd genetics and female reproductive efficiency and propagate offspring from elite sire-dam matings in cattle is embryo transfer. An embryo transfer is composed of two separate procedures. The first half of the procedure is the embryo collection where, in cattle, embryos are collected from superovulated donor cows. The second half is the actual "transfer" where embryos are transferred to recipient cows whose estrous cycles have been synchronized to match those of the donor. In a retrospective report of embryo transfer over the last 40 years, Hasler (2014) cited conception rates for cows used as recipients in nonsurgical transfers for fresh and frozen embryos of 61 and 39%, respectively. When one considers the market value of embryos, an increase by as little as 5% in the percentage of pregnant recipients would generate a significant amount of income, either from selling the additional embryo transfer offspring or the additional pregnant recipients. With an increase in conception rate, there would also be savings in the overall cost associated with maintaining recipients. This savings stems from the interval of recipient maintenance between the day of embryo transfer (day 7 of the estrous cycle for the recipient or 7 days post-insemination relative to the donor) and confirmation of pregnancy (day 45 post-insemination). The cost of recipient maintenance would be wasted on a recipient that did not become pregnant but was maintained as such for 45 days. In a cost analysis of recipient management, Broadbent al. (1991) reported that a 10% improvement in pregnancy rate in the recipients would produce an overall savings of 14% per pregnancy. Clearly, improving recipient pregnancy rate would have financial benefits to producers using embryo transfer.

Between the collection and transfer procedures is an important step where embryos are recovered from the uterine flushing, washed in an embryo culture medium, and evaluated microscopically for stage of development and grade of embryo quality. This is the step where unfertilized ova and degenerate embryos are discarded and embryos can be selected for transfer either immediately to timed recipients or frozen for transfer at a later date. This period also offers a window of time where embryos could be incubated briefly (< 2 h) in a culture medium that enhances or stimulates their development prior to transfer. Regardless of the fate of the embryo, immediate transfer or freezing, it is suspended in a medium referred to as the transfer medium. The transfer medium is the fluid environment in which the embryo is aspirated into the 0.25 cc polyvinyl chloride straw at the end of the washing/grading steps and is what eventually accompanies the embryo into the uterus during the embryo transfer. The current transfer medium has a very simple formulation and is typically composed of a phosphate-buffered saline solution (DPBS), antibiotics and 10% heat-treated cow serum as a protein/nutritive supplement. For freezing embryos, a cryoprotectant, such as glycerol or ethylene glycol, must be added to the transfer medium to prevent freezing damage to the embryo's cells due to ice crystal formation but otherwise there is no difference in composition. Although the nonsurgical embryo collection and transfer procedures have been in use since the late 1970s, the formulation of the transfer medium has not significantly changed since its first use during that period. Given the steps involved in an embryo transfer, the objective of this research was to evaluate conception rates in recipient cows receiving embryos incubated for 16 or 2 h in an enriched embryo culture medium prior to transfer or strawed in a new, restructured transfer medium. The ultimate outcome of this research would be to develop a strategy that would generate greater conception rates in embryo transfer recipients.

Our laboratory has been researching production of the protease, plasminogen activator (PA), by cultured cattle embryos since 1987 (Menino and Williams, 1987). Plasminogen activator secretion is undetectable until the blastocyst stage (Days 6-7 post-insemination), increases during blastocoelic expansion (Days 7-9 post-insemination) and remains elevated throughout and after loss of the zona pellucida or hatching (Days > 9 post-insemination).

High levels of PA production were associated with embryos undergoing vigorous development whereas low levels of PA were produced by poorly developing embryos. Plasminogen activator has many roles in embryo development but its earliest role is in activating uterine plasminogen into the active protease plasmin which functions to soften the zona pellucida and allow the embryo to shed it. The zona pellucida is a protein layer produced by and laid around the ovum while it is still in the ovary. It is an important structure in the fertilization process but is eventually shed on Day 10 post-insemination in the cow. If the embryo fails to escape the zona pellucida in the hatching process, it can neither expand and elongate nor make contact with the uterus and will die within the zona pellucida. When exogenous plasminogen is added to cattle embryos in culture, significantly more embryos successfully shed the zona pellucida and the rate of development is similar to embryos developing in the uterus. Therefore, in the formulation of our enriched culture and transfer media, we included either plasmin or plasminogen. The idea behind including these components was to provide additional plasmin to the embryo for structurally softening the zona pellucida and facilitating zona pellucida loss. Concentrations of plasmin and plasminogen were within physiologic limits for the cow. Besides including plasminogen, the transfer medium was further modified by eliminating the serum component traditionally found in the conventional transfer medium (CTM). Serum is a heterogeneous mixture of a wide array of proteins and many protease inhibitors are present in serum, particularly ones that prevent plasminogen activation and inhibit plasmin proteolytic activity. In place of serum we added 15 mg/ml purified bovine serum albumin (BSA). Bovine serum albumin makes up anywhere from 70-90% of the total protein in the cow's uterine environment depending on stage of the estrous cycle and is widely used as a protein supplement for embryo culture. It also has no effect on conversion of plasminogen to plasmin or plasmin proteolytic activity, unlike the protease inhibitors in serum. For the third and last modification in the formulation of our new enhanced transfer medium (ETM), we replaced the traditional DPBS with a buffered embryo culture medium known as Ham's F-12. Unlike DPBS, which is only a physiologic balanced salt solution, Ham's F-12 is a nutritive medium containing a variety of physiologic biomolecules, e.g., vitamins, sugars and fatty acids, and is the medium of choice for culturing cow embryos.

Materials and Methods

Estrous synchronization and embryo collection: Crossbred beef cows from the Oregon State University Beef Cattle Ranch and dairy cows from the Oregon State University Dairy were used in this project. Donor cows were estrous-synchronized and superovulated following the protocol described in Figure 1. Recipient cows followed the estrous-synchronization protocol described in Figure 1 except the superovulation treatment (FSH injections) was omitted and the second Lutalyse injection was advanced by 12 h.

Estrus detection for the donor and recipient cows commenced 24 h after the second injection of Lutalyse and donor cows were artificially inseminated with one straw of frozen bull semen at 0, 12 and 24 h after onset of estrus. Embryos were collected from donor cows 7 days after estrus onset by non-surgically flushing the uterus with DPBS containing antibiotics and 0.2% heat-treated bovine serum. Embryos were recovered from the flushes and scored for developmental stage and quality using the four rank grading scheme devised by Lindner and Wright (1983). Only embryos having excellent or good quality grades were selected for culture or transfer. Following grading, embryos were washed 4 times in their respective culture or transfer medium.

Experiment 1: Embryos were cultured singly for 16 h in 25-µl microdrops of Ham's F-12 containing 1.5% BSA and 0 or 100 µg/ml plasmin under paraffin oil on 15 x 60 mm plastic tissue culture dishes in a humidified atmosphere of 5% CO2 in air at 39°C. Embryos were recovered from the microdrops, washed in CTM and aspirated into 0.25 cc straws. Embryos were transferred nonsurgically into timed beef cow recipients within +/- 1 day of the donor's estrous cycle.

Experiment 2: Embryos were cultured singly for 2 h in 25-µl microdrops of Ham's F-12 containing 1.5% BSA and 0 or 200 µg/ml plasmin under paraffin oil on 15 x 60 mm plastic tissue culture dishes in a humidified atmosphere of 5% CO2 in air at 39°C. As in Experiment 1, embryos were recovered from the microdrops, washed in CTM, aspirated into 0.25 cc straws and transferred nonsurgically into timed dairy cow recipients.

Experiment 3: Embryos washed in CTM or ETM containing 200 µg/ml plasminogen were aspirated into 0.25 cc straws. Embryos were transferred nonsurgically into timed beef and dairy cow recipients as described in Experiment 1.

Confirmation of pregnancy: To evaluate early embryonic death and confirm pregnancy, 10-ml blood samples were drawn by coccygeal venipuncture from recipients on equivalent gestational days 26, 28, 30 and 32 and assayed for pregnancy-specific protein B (PSPB) using the BioPRYN assay. Pregnancy was reconfirmed by rectal palpation between equivalent gestational days 45-60.

Statistical analysis: Differences between the percentages of pregnant recipients receiving embryos cultured in 0 or 100 µg/ml plasmin for 16 h or 0 or 200 µg/ml plasmin for 2 h were determined by Chi-square analysis. Likewise, differences between the percentages of pregnant recipients receiving embryos strawed in CTM vs. ETM were determined by Chi-square analysis. All analyses were performed using the NCSS statistical software program (Number Cruncher Statistical System; 2000, Jerry Hintze, Kaysville, UT).

Results

Experiment 1: Forty-eight embryo transfers were conducted in crossbred beef cows in Experiment 1. Although more embryos cultured for 16 h in 100 µg/ml plasmin generated pregnancies compared to 0 µg/ml plasmin (50% vs. 38%, respectively), the difference was not statistically significant (Table 1).

Experiment 2: Embryos incubated for 2 h in medium containing 0 or 200 µg/ml plasmin were transferred to a total of 54 dairy cow recipients. Conception rates were identical (52%) for the two groups (Table 1).

Experiment 3: A total of 60 embryo transfers were conducted in dairy and crossbred beef cows in Experiment 3. Conception rates were greater in recipients receiving embryos strawed in ETM containing 200 µg/ml plasminogen compared to the CTM (52 vs. 38%, respectively) however the difference was not statistically significant (Table 1).

Conclusions

Two strategies were assessed for improving conception rates in embryo transfer recipients. The first strategy evaluated was incubating embryos immediately after collection in a modified culture medium containing 0 or 100 µg/ml plasmin for 16 h or 0 or 200 µg/ml plasmin for 2 h prior to transfer. The rationale with this treatment was plasmin exposure would soften the zona pellucida sufficiently to enhance hatching and shedding of the zona pellucida thereby having a positive effect on pregnancy establishment. The 16-h incubation was a really "proof of concept" experiment because the period of culture is too long to merge into current embryo transfer procedures. Nevertheless, conception rate was 12% greater for recipients receiving embryos incubated in 100 vs. 0 µg/ml plasmin. Although the 2-h incubation used a higher dose of plasmin, 200 vs. 0 µg/ml, conception rates were identical (52%). The 2-h incubation may not have provided sufficient exposure to plasmin to exert a positive effect on conception rate.

The second strategy was to develop a new embryo transfer medium that would contain plasminogen for the purpose of softening the zona pellucida and BSA in the place of the commonly used cow serum as the nutrient protein source. Cow serum is rich in protease inhibitors and these inhibitors may interfere with proteases, like PA, that are involved in shedding the zona pellucida. The phosphate buffered saline used in the CTM was also replaced with a more nutritive embryo culture medium, Ham's F-12. Although conception rate was greater by 14% in recipients receiving embryos

strawed in the new plasminogen-containing medium, ETM, compared to the CTM, the difference was not statistically significant, presumably because the number of transfers was low (n = 60) for the difference between the two media. Strawing embryos in ETM can be easily merged into the current transfer procedures and would be the most applicable strategy for improving recipient conception rates. More embryo transfers are planned for the coming year to continue to compare conception rates in recipients receiving embryos strawed in the new ETM containing plasminogen to those strawed in the CTM.

Acknowledgments

The authors wish to thank the Oregon Beef Council for their generous support of this research.

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Table 1. Conception rates in recipient cows receiving embryos incubated for 16 or 2 h in medium containing 0 or 100 µg/ml or 0 or 200 µg/ml plasmin, respectively, or embryos strawed in the conventional transfer medium (CTM) or the new enhanced transfer medium (ETM) containing 200 µg/ml plasminogen.

16 h incubation[a]		**2 h incubation[b]**		**Transfer medium[c]**	
0 µg/ml plasmin	38%	0 µg/ml plasmin	52%	CTM	38%
100 µg/ml plasmin	50%	200 µg/ml plasmin	52%	ETM	52%

[a]48 transfers; beef cows
[b]54 transfers; dairy cows
[c]60 transfers; beef and dairy cows

- **Regimen with Lutalyse**
  - Two injections of Lutalyse with FSH
  - 12-day interval

Day 0 Day 12*
|----------|
Lutalyse Lutalyse

- **FSH:** Total dose of 400 mg where eight 50-mg doses of FSH are administered twice daily, AM and PM, ~12 h apart, over a 4-day period starting 2 days (Day 10) before the second $PGF_{2\alpha}$ treatment (*)

- 4 days of FSH

*2nd Lutalyse
----|-----|-----|-----|----
Days 10 11 *12 13

Figure 1. Double injection Lutalyse and FSH protocol for estrous synchronizing and superovulating donor cows for embryo collections.

BEEF155

Oregon Beef Council Report

Beef Cattle Sciences

Evaluation of Biological Deterrents to Manage Wolf Movements[1]

Lauren Brubaker[2], Lindsay Mehrkam[2], and Monique A. R. Udell[2]

Synopsis

Placement of scat and urine of unfamiliar wolves along a trespass line did not serve as an effective deterrent for captive wolves; cation should be used when employing this deterrent strategy in applied settings.

Summary

Despite the importance of reducing wolf predation on livestock, minimal research has been conducted to assess the effectiveness of existing non-lethal wolf control strategies. The purpose of the current study was to evaluate the effectiveness of creating artificial boundaries using biological stimuli, wolf urine and scat, to delay wolf trespass into protected areas. This was done in captivity, across seven wolf packs, where variables could be carefully controlled and wolf behavior could be observed daily for three months. Each week, one area of each enclosure served as a control where meat was placed behind a trespass line- only colored water was applied as a 'deterrent' along this boundary. Another part of the enclosure severed as the experimental location where either wolf urine, wolf urine + dominant wolf scat, or wolf urine + mixed wolf scat was placed across the trespass line. Locations and stimulus order were counterbalanced. The application of biological stimuli did not significantly reduce wolf trespass into protected areas or increase time to trespass. This result is consistent with research conducted with coyotes in a captive environment, but in contrast to reports of reduced predation in areas treated with biological deterrents in field based settings (e.g. near ranches), therefore more research on this question is needed. Using biological deterrents to control wolf movements may not be reliably effective and therefore should be implemented with caution.

Introduction

The reintroduction of wolves into Oregon has contributed to mortality and stress in livestock resulting in new challenges to the physiological and behavioral welfare and productivity of cattle in this region (Cooke et al. 2013). Wolf predation has also led to new challenges for the Oregon beef industry, including costs associated with the loss of livestock, negative impacts of stressors on the health and reproductive success of cattle, and costs associated with implementing predator control. While ranchers must initially implement nonlethal control measures to receive compensation for losses, many current non-lethal methods are not only costly to ranchers,

1. This document is part of the Oregon State University – 2017 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.\
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but have been understudied by scientists, placing the burden of identifying the most effective antipredator strategies on ranchers themselves. Furthermore, many current nonlethal wolf deterrent methods (e.g., RAG boxes, electric fences, fladry) are only temporarily effective. The temporary effectiveness of these methods has been attributed to habituation to initially novel, artificial (i.e., non-biologically relevant), stimuli not typically present in the wolves' natural environment. Because these methods lack biological relevance, habituation to such deterrents may occur more quickly than if biologically relevant stimuli were used. Some artificial methods, including fladry and electric fences also require that wolves come into direct contact (visual or tactile) with perimeters of cattle grazing areas. This level of exposure to wolves may still result in stress and poor welfare outcomes for the cattle (Cooke et al., 2013) including compromised physiological (e.g., lower calf weight, reduced fertility rates) and behavioral (e.g., increased stress, vigilance, aggression toward working dogs) health, even if a physical attack is prevented. Currently there is no mechanism in place to compensate ranchers for such losses. Controlled scientific studies of wolf deterrents are needed to provide critical information about the effectiveness of different non-lethal control methods and implementation strategies.

Deterrents that consider the natural biology, ecology, and behavior of wolves (biofences) may have advantages over current non-lethal practices that rely on artificial stimuli. Most recently, Ausband et al. (2013) reported that a biofence composed of wolf scat, urine, and simulated wolf tracks were effective at manipulating the movements of free-ranging wolves and reducing livestock predation during the first year of implementation and eliminated livestock predations for the year that the biofence was in place, even though depredations had occurred the previous three years. Given that wolves have evolved a biological predisposition to avoiding the urine, scat, and other signs of unfamiliar wolf presence indicative of territorial establishment, a biofence takes advantage of these instinctual perceptions, increasing the likelihood that wolves across generations will be more likely to avoid such stimuli, in comparison to the individual exposure required for wolves to form associations between artificial stimuli/deterrents and aversive outcomes. The rationale of using urine and feces from unfamiliar wolves to construct a biofence is further supported by numerous observations and long-standing research that wild canids (including coyotes and wolves) avoid these natural indicators of territory maintenance (e.g., Peters & Mech, 1975) by moving away from foreign scent marks of adjacent packs. While prior research is promising, the need for controlled experimental studies of biological deterrents remains to control for proximity and exposure of known wolves to the employed deterrents, formally assess causal relationships between deterrent presence and changes in wolf behavior, control for time from last feeding, and to determine if the source of biological deterrents is important (e.g. scat from a high-ranking alpha vs low-ranking omega) all factors that may influence the likelihood that unfamiliar wolves will not trespass into a territory (Peters & Mech, 1975). Given that ranchers may never be fully compensated for employing nonlethal methods of predator control, the cost-effective nature of biological methods, if effective, would make these methods especially attractive, as urine and scat of unfamiliar wolves can be readily obtained at little to no cost from dozens of captive wolf facilities across the country.

The objective of the current study was to evaluate the relative effectiveness of non-lethal biological deterrents for preventing wolves from approaching prey food stimuli in a controlled setting. Importantly we proposed to test these deterrents on captive wolf populations so that we could obtain more detailed information about long-term effectiveness, under conditions where the same wolves had repeated exposure to the same deterrents (providing information about learning and habituation to deterrents), and so that this information can be acquired without additional cost or losses to ranchers and without risking further exposure of cattle to predator-induced stress. Our

hypothesis is that the presence of biological deterrents (wolf scat and/or urine) will extend the time that it takes captive wolves to trespass into a restricted area to obtain available meat. If this hypothesis is supported, then this would provide additional evidence that biological deterrents could serve as an effective strategy for managing wolf movements in applied settings. However, given mixed findings in prior literature, it is also possible that biological deterrents will not be effective in reducing wolf trespass. Such an outcome would suggest that caution should be taken when utilizing this deterrent strategy in applied settings.

Materials and Methods

Subjects and Setting

Nine captive gray wolves (5 male, 4 female) from the California Wolf Center and eight captive gray wolves (5 males, 3 females) from Wolf Park participated as subjects (n = 17). All procedures took place in familiar enclosures on site, as a parallel to territories in the wild where wolves would be currently active. All wolves had prior experience with consuming livestock and deer carcasses.

Collection of Urine and Fecal Samples for Biological Deterrent Assessment

We collected over 200 pounds of fresh wolf scat from known wolves at our collaborating sites, Wolf Park located in Battle Ground, IN and California Wolf Center located in Julian, CA. Scat was labeled and sorted into 'dominant wolf' and 'mixed status wolf' categories and immediately frozen upon collection. All frozen samples were shipped overnight to the alternative site for experimental testing. Wolf urine (Predator Pee) was obtained from a commercial source advertising the use of the product as a wolf deterrent and antipredator strategy. This allowed us to evaluate a product already being used for this purpose in applied settings. Commercial urine was tested for Leptospirosis (bacteria that can cause severe illness in canines and humans) at the Oregon State University Veterinary Diagnostic Lab to ensure that this product was safe for use in the target settings. All samples came back negative and were therefore deemed safe for use.

Biological Deterrent Assessment

Wolves were exposed to four biological deterrent treatments in paired combinations (See Table 1): Control (water died with food coloring to match the color of the urine), commercially available wolf urine, commercially available wolf urine + unfamiliar dominant wolf scat, commercially available wolf urine + unfamiliar mixed rank wolf scat. Two deterrent areas are present within each enclosure. During the first week- baseline- the control stimulus was placed along all trespass lines, leaving the carcass meat (bait) behind each line unprotected. On all subsequent weeks, one of the three deterrents was applied to one trespass line, to determine if this would deter or slow the removal of fresh bait (meat) by resident wolves over a 7-day period. The alternate deterrent area within each enclosure was set up as a paired control condition. Locations were counterbalanced and facility staff was blind to the testing conditions. Data was collected twice a day indicating the presence, disturbance or absence of the bait behind each trespass line. Trespass events also triggered a motion-activated camera to provide information on the frequency of wolf approaches, other activity in the trespass area (e.g. the presence of crows or staff), and a means for evaluating the behavior of wolves when in the trespass area using an ethogram (see Table 2). This procedure was conducted for four weeks (one round of each experimental condition) at CWC with two wolf packs and conducted for 13 weeks at Wolf Park with five wolf packs (four cycles, replicated across wolf enclosures/packs). Fishers exact tests where used to compare the number of trespasses that had occurred in the experimental vs control areas across the seven wolf packs by the day following each condition setup and the degree to which trespass lines had been breached but the meat only partially consumed (which could indicate hesitation). We also evaluated patterns of wolf behavioral response across from roughly 40,000 videos to further quantify the behavior of

wolves when in the presence of the experimental biofences.

Results

When applied in captivity, the use of unfamiliar wolf scat and urine did not appear to function as an effective deterrent. During the first presentation (the presentation predicted to have the greatest effect) of each deterrent type - commercially available wolf urine, commercially available wolf urine + unfamiliar dominant wolf scat, commercially available wolf urine + unfamiliar mixed rank wolf scat- trespass into the meat baited area had occurred within 12 hours (data collection session 1) in every case across all seven wolf packs, this was no different from the rate of trespass in the control condition (Fishers Exact Test, $p = 1.00$). The degree to which some meat remained behind paired control versus biological deterrent lines the day after setup, which could indicate some degree of approach avoidance conflict even if trespass occurred, was also compared for the first four weeks. Again, no significant difference was found (5 events for control lines, 6 events for pooled deterrent lines out of 21 presentations, Fishers Exact Test, $p = 1.00$). These trends held true across all four cycles of replication conducted at Wolf Park (consisting of an additional 45 paired control vs biological deterrent presentations, 90 presentations total) with only four exceptions: In week 7 one pack did not cross a trespass line consisting of mixed wolf scat + urine for 12 hours, in week 8 one pack did not cross a trespass line consisting of mixed scat + urine for 2.5 days (roughly 60 hours), in week 10 one pack did not cross a trespass line consisting of mixed scat + urine for 24 hours, and in week 10 another pack did not cross a trespass line consisting of mixed scat + urine for 12 hours. In each case the alternate trespass line (e.g. control for the first three, experimental for the last example) was breached by the first data collection session the next day.

Videos for the first six weeks of the study were sorted into wolf positive and wolf negative folders. Wolf positive videos were coded using the ethogram. All total, 37,160 files were collected, with 22,927 videos or photos containing one or more wolves and 14,233 videos or photos containing no wolves. Videos for weeks 3, 4, 5, and 6 (for a total of 12,500 videos and photos) were coded. Data gathered from weeks 1 and 2 was used to create the ethogram. All total, “pacing” was the most common behavior, with 3,450 instances (27.6% of files) of pacing behavior coded. Sniffing and moving away from the deterrent line was the second most common behavior seen, with 509 instances (4.0%). Sniffing and moving past the deterrent line was the third most common behavior seen, with 397 instances (3.2% of files). Fear and conflict behavior was recorded in 389 instances (3.1%), consumption behavior in 105 instances (0.8%), and sniffing in 23 instances (0.2%).

Conclusions

The use of unfamiliar wolf scat and urine as a biological deterrent intended to reduce or slow trespass into protected areas proved ineffective as implemented in this captive setting. Wolves crossed trespass lines made from commercially available wolf urine, commercially available wolf urine + unfamiliar dominant wolf scat and commercially available wolf urine + unfamiliar mixed rank wolf scat into protected areas containing meat just as quickly as they crossed control lines (colored water). While the most promising biological application for increasing time to trespass appeared to be mixed wolf scat + wolf urine, this treatment only had a minimal non-statistically significant effect (12-60-hour delay to trespass) and this only occurred in 3 out of 22 paired presentations of this condition across packs. These findings are consistent with prior research investigating the use of coyote urine as a biological deterrent for coyotes. For example, in 2011 Shivik, Wilson, and Gilbert-Norton found that captive coyotes were not deterred by the presence of unfamiliar coyote urine, and in fact spent more time in areas with this scent when compared to control areas. Therefore, caution should be taken when using biological deterrents in applied settings, including protection of livestock, as such approaches may not deter the approach of predators, especially

when used alone. This is especially concerning given that biological materials, such as wolf urine, are already publically marketed and widely advertised for this purpose. However, given that field based research has suggested that biological deterrents may be effective in some contexts (Ausband et al., 2013) more research should be conducted before the use of biological deterrents is ruled out completely, as it is possible that captive wild canids respond differently to the presence of biological deterrents when compared with their free-living counterparts.

Acknowledgments

This research study was financially supported by the Oregon Beef Council. We also with to thank the staff and volunteers of California Wolf Center, Julian, CA and Wolf Park, Battle Ground Indiana for allowing us to use their facilities and for the many hours of time they dedicated to this project, special thanks to Erin Hunt of CWC and Karen Davis of Wolf Park without whom this research would not have been possible..

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Week #	Deterrent Area 1	Deterrent Area 2
1	Control	Control
2	Control	Wolf Urine
3	Urine & Dominant Scat	Control
4	Control	Urine & Mixed Scat
5	Wolf Urine	Control
6	Control	Urine & Dominant Scat
7	Urine & Mixed Scat	Control
8	Control	Urine & Dominant Scat
9	Wolf Urine	Control
10	Control	Urine & Mixed Scat
11	Urine & Dominant Scat	Control
12	Control	Wolf Urine
13	Urine & Mixed Scat	Control

Table 1. Schedule of experimental conditions. Each week one experimental and one control deterrent was presented in each wolf pack's enclosure with locations counterbalanced across weeks.

Scent Rolling	Rubbing the ground with face, neck, shoulder or back in repetitive motions
Pacing	Continuous locomotive movement from left to right in a straight line pacing the feet exactly in the same position each way
Consumption behavior	Taking something into the mouth where part or all of the item is not visibly spit out again.
Sniffing	Placing nose to the ground or visibly taking air in through the nostrils
Fear/conflicted behavior	Any of the following when the wolf sniffs or comes into contact with the trespass line: crouching, long reach with head toward food while trying to keep body away from line, ears back, hackles up, quick movement away from line, dropping food to get out of trespass area quickly.

Table 2: Ethogram of the behaviors coded.

Oregon State University

Oregon Beef Council Report

Beef Cattle Sciences

Progress Reports – Animal Sciences [1]

Feeding essential fatty acids to late-gestating cows to optimize performance and health responses of the offspring

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Project Objectives: Determine the effects of omega-6 fatty acid supplementation to pregnant beef cows during the last trimester of gestation on epigenetic responses, growth, health, and carcass characteristics of the offspring.

Project Start Date: December of 2017
Project Completion Date: July of 2019

Project Status: Ninety-six multiparous Angus × Hereford cows will be ranked by body weight, body condition score, and allocated to 24 groups (4 cows/group) at the end of their second trimester of gestation (day 0 of the study). To ensure that all cows are in the same stage of gestation and offspring share similar genetic background, we will assign to the study only cows that became pregnant to an estrus synchronization + fixed-time AI using sire from a single bull. Groups will be randomly assigned to receive: 1) 200 g/day of a omega-6 fatty acid supplement, or 2) 200 g/day of a non-essential fatty acid source (to serve as control). Options for the omega-6 supplement include commercial rumen-protected sources such as Essentiom (Arm & Hammer Animal Nutrition, Princeton, NJ) or Prequel (Virtus Nutrition, Corcoran, CA), as well as natural sources such as canola and soybean byproducts. However, using rumen-protected sources are preferred to facilitate feeding in a commercial cow-calf scenario, and ensure that omega-6 fatty acids are not being broken down in the rumen. Hence, we propose the use of Essentiom as the omega-6 source due to its greater ruminal stability, and EnergyBooster (Milk Specialties, Eden Prairie, MN) as the saturated control fatty acid source.

Treatments (200 g of Essentiom or 200 g of EnergyBooster) will be mixed with 400 g/cow of soybean meal and delivered individually to groups from day 0 of the experiment (end of second trimester of gestation) until calving. Cows from both treatments will also receive 35 pounds daily of alfalfa-grass hay during this period.

Sampling

Cows

On day 0 of the experiment and immediately upon calving, cow BW and BCS will be recorded and blood samples collected for plasma fatty acid profile (gas chromatography). Colostrum samples and the expelled placenta will also be retrieved concurrently with blood collection when feasible (target = 30 samples/treatment) for fatty acid profiling and immunoglobulin G concentrations (colostrum samples only). After calving, treatment administration will be terminated, cow-calf pairs will be removed from their experimental groups and assigned to the nutritional and general management EOARC (which does not include essential fatty acid supplementation).

1. This document is part of the Oregon State University – 2017 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.\

Approximately 45 d after calving, cow milk production will be evaluated via weigh-suckle-weigh technique. More specifically, calves will be separated from their dams for 8 h and allowed to suckle for 30 min. Milk yield will be calculated as the difference between pre- and post-suckling calf BW multiplied by 3. Milk samples will be collected from 30 cows/treatment and evaluated for milk composition (Dairy One Lab) and plasma fatty acid profile.

Offspring – birth to weaning

Immediately after birth, calf BW will be recorded and blood samples collected for plasma fatty acid profiling from all calves, whereas *longissumus* muscle samples will be collected from a subsample of calves (n = 30/treatment). Muscle samples will be analyzed for expression of cell differentiation and growth-related genes. Muscle samples will also be analyzed for global DNA methylation (Methylflash Methylated DNA Quantification Kit; Epigentek, Farmindale, NY) to search for evidences of epigenetic responses in muscular and intramuscular DNA to treatments applied on their dams. Twenty-four hours after birth and at the time of the weigh-suckle-weigh, blood samples will be collected from another subsample of calves (n = 30/treatment) and analyzed for immunoglobulin G concentrations (Bethyl Laboratories, Montgomery, TX).

Offspring – after weaning

Approximately 7 months after birth, calves (steers and heifers) will be weaned and maintained in a single pasture for a 45-day preconditioning. During this period, calves will be managed as a single group in meadow pastures harvested for hay the previous summer, and receiving alfalfa-grass hay for ad libitum consumption. Calves will be dewormed and receive vaccination against *Clostridium* and respiratory pathogens on day 15 and 43 of the preconditioning period. Blood samples will be collected at weaning, and on days 1, 3, 5, 7, and 10 of preconditioning and for analysis of plasma haptoglobin, cortisol, and IGF-I to evaluate innate immunity and growth physiology parameters. Blood samples will also be collected on days 15, 22, 29, 36, and 43 of preconditioning and analyzed for antibody titers against respiratory pathogens to assess vaccine efficacy.

After preconditioning, calves will be transferred to a commercial feedlot (Beef Northwest; Boardman, OR), where they continued to be managed as a single group until slaughter at a commercial packing facility (Tyson Fresh Meats Inc., Pasco, WA). Upon arrival at the feedlot, steers will be implanted with Revalor IS (Merck Animal Health, Kenilworth, NJ) and heifers were implanted with Revalor IH (Merck Animal Health) upon arrival. Approximately 4 months after feedlot entry, *longissimus* muscle samples will be again collected from a subsample of calves (n = 30/treatment) and analyzed for mRNA expression of growth-related genes, and global methylation in muscular and intramuscular DNA.

During preconditioning and feedlot, calves will be observed daily for bovine respiratory disease symptoms according to the DART system (Zoetis, Florham Park, NJ), and treated accordingly when symptoms are observed. Upon slaughter, hot carcass weight will be collected and finishing final BW estimated based on 63% dressing percentage. After a 24-h chill, trained personnel will assess carcass backfat thickness at the 12^{th}-rib and longissimus area, whereas all other carcass measures were recorded by a USDA grader. Preconditioning will be ADG determined using BW obtained at weaning and upon feedlot lot arrival. Feedlot ADG will determined using BW values obtained upon feedlot arrival and final finishing BW estimated from hot carcass weight.

Impacts of estrus expression and intensity during an estrus synchronization + artificial insemination protocol on parameters associated with fertility and pregnancy establishment in beef cows

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Project Objectives: Evaluate the impacts of estrus expression and intensity during an estrus synchronization + AI protocol on parameters associated with fertility and pregnancy establishment in beef cows.

Project Start Date*:* April 2018

Expected Project Completion Date: December 2018

Project Status: Three hundred multiparous, lactating Angus cows, averaging 70 to 90 days post-partum, will be assigned to an estrus synchronization + AI protocol: 100 µg of GnRH (Factrel; Zoetis, Florham Park, NJ, USA) plus a controlled internal device release (CIDR) containing 1.38 g of progesterone (Zoetis), followed in 7 days with 25 mg of prostaglandin F2α (Lutalyse; Zoetis) and CIDR removal, followed in 60 h by a second 100 µg injection of GnRH and fixed-time AI. All cows will be inseminated by the same technician, with semen from a single Angus bull.

At the beginning of the synchronization protocol, 1 pedometer (HJ-321; Omron Healthcare, Inc., Bannockburn, IL) will be attached on top of the right shoulder of each cow, placed inside a polyester bag (Heat Watch II; Cow Chips, LLC, Manalapan, NJ) as validated by our research group (Schubach et al., 2016). At the time of prostaglandin F2α injection and CIDR removal, cow physical activity (steps per day) will be assessed and considered basal activity, whereas estrus detection Estrotect patches (Rockway Inc., Spring Valley, WI, EUA) will be attached to tail base of each cow. At the time of AI, estrus detection patches and pedometers will be evaluated. Cows will be classified as estrus expression or not according to activation of the Estrotect patch. In addition, within cows that expressed estrus, cows will be classified as high-intensity estrus (50% of population with higher physical activity) or low intensity estrus (50% of population with less physical activity) as suggested by Silper et al. (2015) in dairy cows.

Sampling

Ovarian ultrasonography will be performed at the time of AI and 7 days post-AI to determine ovulation and synchronization rates, as well as follicular diameter (at AI) and corpus luteum volume (7 days after AI). Blood will be collected concurrently with ovarian ultrasonography exams and analyzed for plasma estradiol (AI) and progesterone (AI and 7 days later) concentrations. On day 21 post-AI, blood will be collected in RNA-specific tubes and subsequently analyzed for mRNA expression of interferon-stimulated genes, which are indicators of early embryo presence and growth. On day 30 post-AI, pregnancy diagnosis will be performed via ultrasonography, and blood will be collected for the analysis of plasma concentrations of pregnancy-associated glycoproteins for embryo viability assessment. Final pregnancy rates to AI will be determined according to calving rate and date, given that cows will be exposed to mature Angus bulls 15 days after AI which allows differentiation among AI-sired or bull-sired calves.

Identification of cyanobacterium responsible for mass cattle deaths in Lake County, June 2017

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Project Objectives: Identification of cyanobacterium responsible for cattle deaths in Lake County
Project Start Date*:* June 2017

Expected Project Completion Date: June 2018

Project Status: On 19th June, 2017, steers on a ranch 10 miles west of Lakeview, OR, began dying on the margins of Junipers Reservoir, an impoundment of 175 acres on the ranch. Most deaths accumulated rapidly, but additional cattle died over the following two days. An intense algal bloom had accumulated at the south end of the reservoir (X in Fig. 1), concentrated by persistent northerly wind. Cattle had been using the reservoir for drinking water and some were seen to have accumulated blue-green scum on their legs. In the end, thirty-one 14-month-old steers, worth some $50,000, had died.

OSU Extension Agent Peter Schreder and Lakeview Animal Hospital veterinarian Rod Ferry became involved in assessing the cause of the deaths. I was also involved in providing advice within a day of the first deaths. Samples of the reservoir water and rumen contents of a rapidly deceased steer were submitted to the California Animal Health and Food Safety Laboratory at UC-Davis Veterinary Diagnostic for testing for the presence of the cyanobacterial liver toxin microcystin, the presumed cause of death. Extremely high levels of microcystin-LR were detected in the reservoir water (3000 ppb; the Oregon recreational guideline value for microcystin is 10 ppb) and in the rumen contents (7100 ppb). Other samples were sent to the OSU Veterinary Diagnostic Laboratory, where Dr. Rob Bildfell observed major liver damage with post necrotic collapse and severe diffuse hepatic vacuolation.

Three samples were obtained by my laboratory for DNA analysis to detect the cyanobacteria present. A reservoir water sample of the scum material (sample ID JUN01) was delayed in transit and suffered substantial degradation. Nevertheless, large numbers of akinetes (spore-like cells) indicative of abundant Anabaena were observed microscopically; akinetes are not produced by the Microcystis, which is most commonly associated with microcystin production. Comprehensive identification of all cyanobacteria that were present initially in the sample was not possible because of degradation. A second lake water sample (JUN03) that had been stored frozen except during thawing to allow samples to be aliquoted, arrived via the Davis analytical laboratory. That same sample had been used for toxin analysis. Due to freeze-thaw treatment, this sample was also not useful for microscopic identification of cyanobacteria, although once again the robust akinetes were present. The third sample was a rumen sample from a deceased steer, also transferred from Davis, which had registered high microcystin concentrations.

We have processed the JUN01 and JUN03 samples for comprehensive DNA sequencing, creating a "metagenomics" library that is meant to represent all of the DNA in a sample. Successful libraries were constructed for each sample. The JUN03 sample had substantial numbers of DNA sequence "reads" that correspond closely to a large cluster of genes (mcy genes) that have been described in Anabaena sp. 90 and shown to be responsible for the production of microcystin. The Anabaena mcy genes are distinct from related mcy genes that have been described in other cyanobacteria, specifically Microcystis and Planktothrix. We have also detected the presence of abundant Anabaena-specific genes encoding the cyanobacterial marker proteins phycocyanin a and b.

Our preliminary analysis is consistent with a strain of Anabaena being the major microcystin producer. We are looking more closely to see if other producers of microcystin (or other toxins) were present in low levels, and specifically whether any Microcystis (the most common microcystin producer) was present. We will also extend these analyses to the JUN01 sample.

To complete the study, we will use polymerase chain reaction (PCR) to look for the same mcy genes in the rumen sample. Our preliminary results suggest that Anabaena was the toxin producer, and we expect to see the same Anabaena genes in all three samples. Our results will be submitted for publication together with a case report of the intoxication incident and the results of post-mortem and toxin analysis. Unless our further analyses

uncover new details, the report will serve the first warning that Anabaena blooms can be the source of high levels of microcystin and are a potential threat to farm stock.

Graduate student Lindsay Collart has been conducting these analyses.

Does Feeding Selenium Fertilized Alfalfa Hay for Eight Weeks Decrease Gastrointestinal Parasite Load in Weaned Beef Calves?

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Project Objectives: The objective of the proposed study is to determine if feeding Se-enriched alfalfa hay to weaned beef calves decreases gastrointestinal parasite load, or the percentage of parasites that are *Haemonchus contortus*.

Project Start Date: Funded Fall of 2016
Expected Project Completion Date: Winter of 2018

Project Status: Alfalfa hay was enriched with Se by mixing inorganic Na-selenate with water and spraying it onto regrowth of an alfalfa hay field at an application rate of 89.9 g Se/ha immediately after the second cutting of hay in July 2017. The application rates were chosen based on a previous study. Third-cutting alfalfa hay was harvested in October 2017 and then analyzed for nutrient and Se content. A Penn State forage sampler was used to take multiple cores from random bales in each hay source (control or 89.9 g Se/ha) prior to beginning the feeding trial. Core samples were blended and representative samples were selected for analysis. Alfalfa hay samples were submitted to commercial laboratories for analysis. The Se concentration of alfalfa and grass hay were determined using inductively coupled argon plasma emission spectroscopy (ICP-MS).

Weaned beef calves (30 steers), approximately 8 months of age, were utilized from the Oregon State University beef ranch, Corvallis, OR. Body weights at baseline ranged from 265 to 370 kg (mean 329 kg), and body condition scores ranged from 6 to 7 (1 to 9 scale). Routine farm management practices, including vaccinations and deworming, were the same for all treatment groups. Calves were blocked at baseline by parasite load and body weight and then assigned to one of 2 treatment groups (control or Se-enriched hay) with 15 calves each. Ear tags were used to identify calves. Calves were then placed by treatment group into pens (3 pens of 5 calves/treatment group). Three pens of each treatment are needed for replication (n=3). Pen is the experimental unit and multiple animals within the pen provide multiple measurements of treatment response. Group 1 (No-Se forage control): 3 pens of calves will be fed non-Se fortified alfalfa hay as the major portion of the ration. Group 2 (High-Se forage): 3 pens of calves will be fed alfalfa hay fertilized with 89.9 g Se/ha. Calves will be maintained on their respective diets for 8 weeks before sale to a commercial feedlot.

Calves are/will be fed 85% alfalfa hay and 15% grass hay twice daily. The ration was formulated for growing beef calves in the 260 to 380 kg weight range to achieve a target average daily gain of 0.5 kg/day. Calves were transitioned to their respective alfalfa hay sources over a 12 day period. Alfalfa hay was fed as follows: 0.68 kg/head/day 1; 1.14 kg/head/day 2; 1.59 kg/head day 3; 2.27 kg/head/day 4; 2.95 kg/head/day 5; 3.41 kg/head/day 6; 3.86 kg/head/day 7, 4.32 kg/head/day 8, 4.77 kg/head/day 9, 5.23 kg/head/day 10, 5.68 kg/head/day 11, and 5.91 kg/head/day 12. Grass hay was added to achieve a total hay intake of 5.77 kg/head on days 1 through 5; 6.48 kg/head on days 6 through 12; and thereafter as needed to provide all the hay they wanted for consumption yet with minimal wastage. Prior to this study, calves had free-choice access to a mineral supplement in loose

granular format containing 120 mg/kg Se from sodium-selenite (Wilbur-Ellis Company, Clackamas, OR). During this feeding trial, mineral supplement without Se is provided.

Blood samples were/will be collected from the weaned beef calves, at baseline and after 2.5, 5.5, and 8.5 weeks of alfalfa hay consumption for measurement of whole-blood Se concentrations. Selenium concentrations will be determined by a commercial laboratory (Utah Veterinary Diagnostic Laboratory, Logan, UT) using an ICP-MS method. Fecal samples were/will be collected at baseline and at the end of the 8 week feeding period. Feces will be collected directly from the rectum for fecal flotation to determine the number of ova/gram of feces (McMaster's technique). This test will be performed in the Veterinary Diagnostic Laboratory at Oregon State University. In addition, feces will be submitted for an ELISA assay for determination of *Haemonchus contortus* incidence. Results will be used to determine the effectiveness of Se-enriched alfalfa hay in raising whole-blood Se concentrations in weaned beef calves and to correlate whole-blood Se concentrations with efficacy of parasite control.

An in vivo-in vitro hybrid system to perform nutrigenomic studies in cattle: validation using peripartum cows.

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Project Objectives: To develop an *in vivo-in vitro* hybrid system and test the hypothesis that NEFA around parturition activate PPAR in liver and mammary cells of cattle and affect expression of genes. The long-term goal is to develop an *in vivo-in vitro* hybrid approach to carry out nutrigenomic studies in cattle.

Project Start Date: September of 2016
Project Completion Date: December of 2017

We are submitting this project as progress report and not final report

Project Status: The delay of **the start of the experiment is twofold: 1)** The *in vivo* part of the experiment only started in August 2017, since we had to get approval from our IACUC to perform liver biopsies we were able to do it by inserting this project with another project that only started in August due to several circumstances (originally should have started in February 2017). Despites the delay on starting the experiment, we have successfully develop protocols to accomplish the trial. Hepatic cells were successfully isolated using samples obtained from liver biopsies. However, due to typically small sample size (300 mg) the total cellular yield became a constraint. During the last few weeks, the graduate student leading this project (Sebastiano Busato) is implementing a novel two-step perfusion technique that has so far increased the total yield between 5 and 10-fold. In order to validate the isolation technique, appropriate characterization is in order; for this purpose, albumin and urea concentration in the media, as well as the expression of liver specific genes, will be assessed.

We were also successful in isolating Mammary Epithelial Cells (MEC) from 1L milk samples were we obtained a considerable amount of cells, which are currently being cultivated.

A solid method to isolate plasma and serum NEFA, using aminopropyl SPE cartridges, is being assessed and is expected to achieve good recovery of the free fatty acid fraction in the blood. This will allow to treat the cells with the very same fatty acids present in the blood around parturition, and will provide a more accurate understanding of how they affect PPAR activity in the liver and the mammary gland.

In the following months, PPAR activity in both types of cells will be tracked using a synthetic fluorescent reporter, as previously described.

Use of Platelet Rich Plasma for the Treatment of Subclinical Endometritis in Beef Heifers

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Project Objectives: Calving difficulty in first-calf heifers is an important economic issue in the beef industry not only because of the risks to the calf, but also because of the effects of impaired fertility following delivery on the mother. While efforts are made to minimize factors that contribute to calving difficulty (e.g. using expected progeny differences (EPDs) for lower birth weight or improved calving ease), the overall prevalence of beef heifers needing assistance is still 10-20%. Failure to conceive at second mating is the most common reason for heifer attrition. About 4% of heifers are culled at second mating after being diagnosed non-pregnant and about 2.3% are carried over as non-pregnant 3-year-old heifers.

During calving, the uterus is exposed to bacterial contamination, which can cause inflammation of the uterine lining (referred to as "endometritis"). If calving is prolonged, the severity of bacterial contamination and endometritis increases. In healthy cows following a normal calving, bacterial contamination and endometritis are spontaneously cleared within two weeks, which can be confirmed using bacterial culturing endometrial cytology methods (e.g. reduction in the number of neutrophils). However, about 40% of cows fail to spontaneously clear bacterial infections and/or have prolonged inflammatory conditions that persist more than 50 days postpartum, which severely affects fertility. It is important to note that these cows do not show any external evidence that there is a problem (referred to as "subclinical"). Ricci and coworkers (2015) reported that only 13% of beef heifers with subclinical endometritis were pregnant within 130 days postpartum. Subclinical endometritis reduces pregnancy rates in beef heifers by 16%, and despite this, research in this area has been poorly investigated.

In cows, many therapeutic agents and procedures have been used to treat endometritis, including systemic or intrauterine administration of antibiotics, or administration of PGF2α (Lutalyse®) or its analogue (Estrumate®). The efficacy of most of these treatments is low, while the costs (labor and drugs) are high. It would be highly desirable for beef producers to have a specific treatment aimed at reducing uterine inflammation that would not result in meat residues and could be administered by an artificial insemination (AI) technician.

Platelet rich plasma (PRP) is an emerging therapeutic application in tissue regeneration because of its enrichment with growth factors and anti-inflammatory properties. Platelet rich plasma is known to accelerate the healing process in human medicine and has been used in facial surgery, muscle and tendon repair, and reversal of skin ulcers. In veterinary medicine, it has been mainly used for promoting equine tendon repair, but there are some reports of its use in intestinal wound healing in pigs and in skin wound healing in dogs. In dairy cattle, PRP has been used to treat mastitis, repeat breeders, and to increase embryo production in embryo transfer programs.

The objective of the proposed research is to investigate the effect of PRP on the resolution of subclinical endometritis in beef heifers. We hypothesize that intrauterine treatment with PRP will decrease endometrial bacterial and white blood cell counts, cervical discharge, and uterine diameter, and increase subsequent first cycle pregnancy rates.

Project Start Date*:* April 2017

Expected Project Completion Date: March 2020

Project Status: Platelet-rich plasma (PRP) collection: One liter of whole blood has been collected from a donor Jersey cow in good health, tested free from infectious diseases (e.g. Johne's disease, bovine leukemia virus) and prepared using the double centrifugation method as reported by Lange-Consiglio and colleagues (2014). Briefly, after a surgical scrub preparation of a few centimeters of skin around the jugular vein, blood was collected into blood bags containing citrate phosphate-dextrose-adenine (CPDA-1) using the 16 gauge needle provided with the bags. The bags were transported at 4°C to the laboratory and immediately processed. All separation steps were performed in a horizontal laminar flow hood under aseptic conditions. To prepare the PRP, the blood was transferred into sterile 50 mL tubes and centrifuged at 400xg for 30 minutes at 4°C (with the centrifuge brake turned off). The upper layer was carefully aspirated and transferred to new 50-ml tubes and centrifuged again at 1,500xg for 10 minutes at 4°C to obtain the platelet pellet and the poor platelet plasma (PPP)

on the upper layer. Two-thirds of the volume of PPP was aspirated for later use and the platelet pellet was mixed then in the residual PPP volume to perform a platelet count. The PRP was then diluted with additional PPP to make a final concentration of 1×109 platelets/ml. The PRP was frozen at −80°C and thawed at 37°C three times to use to allow the release of platelet-derived growth factors. The PRP was then tested for aerobic and anaerobic bacterial contamination to verify its sterility. Finally, the PRP was aliquoted in 10 ml ready-to-use syringes and stored at −20°C.

Endometrial sampling: Two cows and two heifers that calved late in the season were used to perfect the endometrial sampling technique. Two to three weeks after calving, each animal was restrained in a chute. The reproductive tract was transrectally palpated and examined using a high-resolution ultrasound scanner equipped with a 7.5 MHz linear array probe (M5 Digital Ultrasound, Mindray). Longitudinal images of each uterine horn will be digitally captured just beyond the bifurcation to quantify the cross-sectional diameter and to determine the endometrial contrast and homogeneity. The base of tail, perineum and external genitalia were thoroughly cleaned and disinfected before a vaginal speculum was inserted and the presence, amount, and color of cervical mucus was recorded. Next, an endometrial cytology was collected using a cytobrush. The presence of red blood cells, white blood cells, and endometrial cells was quantified and recorded. Cells from a second cytobrush were recovered by rinsing the end of the brush with 0.9% sterile saline and saved for gene expression preliminary studies. The results from these initial studies were presented by an undergraduate student in the College of Agricultural Sciences at the Oregon State University Celebrating Undergraduate Excellence Poster Fair in May 2017 and at the Oregon State University College of Agricultural Sciences Experiential Expo in October 2017. A copy of the presented abstract can be found below.

Natural Resolution of Postpartum Endometritis in Beef Cattle

Ellie Puttman[a], Michelle Kutzler[b]

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Postpartum endometritis is an important cause of infertility in beef cattle.[1] Many methods have been used to diagnose endometritis. The objective of this research was to evaluate the natural resolution of postpartum endometritis using three different methods: total uterine thickness, cervical discharge score, and endometrial cytology. The hypothesis was that each method would show a significant reduction in endometritis between the early and later time point. Four crossbred beef cows were restrained in a chute and examined at 17±3 and 31±3 days postpartum. Each exam included: transrectal palpation and ultrasonography of the cervix and uterus, vaginal speculum exam, and endometrial cytology (via cytobrush). Total uterine thickness was determined by ultrasonography using the average cross-sectional diameter of both uterine horns. A post-cervical vaginal discharge score of 1 (no mucus) to 5 (copious purulent mucus) was adapted from de Boer et al (2015).[2] Cytology slides were stained with Diff-Quik® and 200 nucleated cells were counted at 400X magnification to determine the percentage of polymorphonuclear cells (%PMNs) and macrophages (%MACs). Results were reported as mean ± SD. A Student's t test was used to compare results from the two time points. The total uterine thickness did not differ at 17 ± 3 and 31 ± 3 days postpartum (3.02 ± 0.23 cm compared to 3.3 7± 0.81 cm, respectively; p=0.258). However, the post-cervical discharge score decreased from 1.67 ± 1.15 to 0 ± 0 at 17 ± 3 and 31 ± 3 days postpartum, respectively (p=0.033). In addition, the %PMNs decreased from 12.5 ± 5.27% to 2 ± 1.32% at 17 ± 3 and 31 ± 3 days postpartum, respectively (p=0.014). On the other hand, the %MACs did not differ 6.17 ± 2.08% and 3.83 ± 3.21% at 17 ± 3 and 31 ± 3 days postpartum, respectively (p=0.175). These results are comparable to those from a previous study by de Boer and colleagues (2015)[2] that only evaluated endometrial cytology at 0 and 42 days postpartum. This is the first study to compare the natural resolution of postpartum endometritis in beef cattle at two time points prior to breeding prior to the voluntary waiting period (42 days postpartum). Future studies will evaluate if intrauterine treatment with platelet-rich plasma will further improve the resolution of postpartum endometritis in beef cattle. This research was generously supported by the Oregon Beef Council.

[1]Sheldon, et al. *Theriogenology* 2006;65:1516-1530.

[2]de Boer, et al. *Theriogenology* 2015;83:1514-1524.

Development of an enhanced cattle embryo transfer medium to improve pregnancy rates in embryo transfer recipients

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Project Objectives: The objective of this ongoing study is to compare conception rates in recipient cows receiving embryos strawed in either the conventional embryo transfer medium or a new, modified transfer medium containing 200 µg/ml bovine plasminogen.

Project Start Date: December 2015
Expected Project Completion Date: September 2018

Project Status: Since January 2016, we have performed 60 embryo transfers comparing our new synthetic transfer medium (ETM) to the conventional medium (CTM) commonly used in the industry. Current conception rates are 52% vs. 38% for recipients receiving embryos strawed in ETM vs. CTM, respectively, however the difference is not statistically significant. We are planning to conduct an additional 30 transfers by September 2018.

Peripartal vitamin E injections prevent diseases in dairy cows

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Project Start Date: Spring 2016
Project Completion Date: March 2019

Project Status: We have been working for the last 1.5 years on the project “Peripartal vitamin E injections prevent diseases in dairy cows”. Initial progress for the project was good but then we ran into unforeseen staffing issues. We worked hard over the last year to resolve the staffing issue and trained several undergraduate students so that they were trained to take over the project once they are graduated in Spring 2017. One of the students took over in October 2017 the project so that we can start in late Spring early Summer 2018 the animal trial, as this is the time with the highest incidence of transition cow diseases. Hoping to finish the study by the end of the summer break 2018, we will work in Fall and Winter 2018/2019 on the chemical analysis, so that we have the results ready for submission at the beginning of March 2019, so that we can present them to you and at the regional and national meetings in the Summer 2019.

Oregon State University

BEEF157

Oregon Beef Council Report

Beef Cattle Sciences

Techniques to improve seeding success of forage kochia in exotic annual grass invaded sagebrush rangelands[1]

Dustin D. Johnson[2], Kirk Davies[3], Matthew Madsen[4], and Travis Miller[5]

Synopsis

Seeding forage kochia during winter (February) generally increased its initial establishment compared to fall seeding (November). The performance of year-old seed that had been properly stored was not markedly different compared to freshly harvested seed when both were seeded during the winter.

Summary

The objectives of this study were to determine which seeding techniques are appropriate for enhancing the establishment of forage kochia, a promising revegetation species for sagebrush rangelands prone to invasion by exotic annual grasses. Specifically, we evaluated three seeding methods, two timings of seeding, and the efficacy of stored versus freshly harvested at two sites with five replicated randomized blocks per study site. Our findings indicate shallow drill seeding forage kochia during late winter (February) will generally increase its initial establishment success over broadcast seeding in winter and broadcast or drill seeding during the fall. In addition, we did not observe a marked difference in the performance of year-old seed that had been properly stored compared to freshly harvested seed when both were seeded during the winter. Lastly, seed enhancement treatments designed to improve germination and emergence did not improve establishment of forage kochia compared to shallow drill seeding in the winter, suggesting seed germination and emergence may not be limiting factors for establishment if appropriate planting timing and techniques are employed.

Introduction

Revegetation of arid sagebrush rangeland invaded by exotic annual grasses is arguably the most pressing management challenge that contemporary rangeland managers face. This challenge is particularly acute on warmer and dry sagebrush rangelands. Not only are these rangelands most susceptible to invasion by exotic annual grasses, but, because of inherently low and erratic precipitation and extremes in temperature, they are also the most difficult to rehabilitate once degradation has occurred. The relatively harsh environment associated with these areas greatly limits the number of revegetation species that can be

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successfully established. Crested wheatgrass has been effectively used to revegetate exotic annual grass invaded rangeland (Davies 2010), whereas other species have not consistently established (Davies and Johnson 2015, OBC-funded research project).

The challenge of revegetating arid sagebrush rangeland is particularly acute for livestock managers who rely on these rangelands to provide a reliable proportion of their operation's forage base. Invasion by exotic annual grasses and associated degradation of rangeland productivity greatly limits forage availability and quality. Exotic annual grasslands can provide a sufficient quantity and quality of early season forage, but often fail to produce the availability of quality forage needed to meet the nutritional demands of livestock by early-summer through late fall. In addition, although crested wheatgrass has been successfully used to revegetate rangelands invaded by exotic annual grasses, these seedings are typically of a deficient quality by the middle to late summer to adequately meet the nutritional requirements of grazing livestock. Forage kochia is a revegetation species that could hold promise for improving the diversity, productivity, and later-season quality of rehabilitated arid sagebrush rangelands. Forage kochia can be used to significantly increase later season protein availability to wildlife and livestock when cool season grasses are dormant (Schauer et al. 2004).

However, efforts to establish forage kochia have produced inconsistent results in the northern Great Basin that may be related to seeding method (broadcast vs. drill), timing (fall vs. late winter/spring), and/or quality degradation of stored seed.

The objectives of this study were to determine which seeding techniques are appropriate for enhancing the establishment of forage kochia. Specifically, we evaluated three seeding methods, two timings of seeding, and the efficacy of stored versus freshly harvested seed.

Materials and Methods

Two study sites in central Harney County, Oregon were selected for inclusion in the study during 2014. Study sites were separated by 26 air miles and located in Loamy 10-12" Ecological Sites. The Loamy 10-12" PZ Ecological Site was selected because it comprises a large proportion of sagebrush rangeland for which forage kochia may be applicable as a revegetation species. We were also interested in evaluating the revegetation efficacy of forage kochia in areas invaded by exotic annual grasses, therefore, study sites were also selected because current vegetation is dominated by cheatgrass with a lesser amount of crested wheatgrass. Existing vegetation at each site was treated with glyphosate during the summer and then burned with a trailer mounted propane torch in the fall to prepare a seedbed for seeding treatments. The following seeding treatments were applied to 6 X 18 ft plots arranged in a randomized block design, with 5 replicated blocks per study site:

1. Fall broadcast of year-old seed
2. Fall drill of year-old seed
3. Fall broadcast of pellets made with year-old seed
4. Fall drill of pellets made with year-old seed
5. Winter broadcast of year-old seed
6. Winter drill of year-old seed
7. Winter broadcast of pellets made with year-old seed
8. Winter drill of pellets made with year-old seed
9. Winter broadcast of freshly harvested seed
10. Winter drill of freshly harvested seed
11. Winter broadcast of pellets made with freshly harvested seed
12. Winter drill of pellets made with made with freshly harvested seed
13. Unseeded control
14. Fall drill of year-old seed with a hydrophobic coating

Year 1 fall and winter seeding treatments were conducted during early November 2014 and early February 2015, respectively. Forage kochia was seeded at 2 lbs/ac pure live seed in all plots that received a seeding treatment. Vegetation cover and

density by species were measured during the summers of 2015and 2016 in each Year 1 treatment.

In Year 2, the following seeding treatments were replicated at each study site:

1. Fall broadcast of year-old seed
2. Fall drill of year-old seed
3. Winter broadcast of year-old seed
4. Winter drill of year-old seed
5. Winter broadcast of freshly harvested seed
6. Winter drill of freshly harvested seed
7. Unseeded control

Year 2 fall and winter seeding treatments were conducted during early November 2015 and mid-February 2016, respectively. Forage kochia was seeded at 2 lbs/ac pure live seed in all plots that received a seeding treatment. Seeds or pellets were planted to a depth of approximately 0.5-inch in plots that received a drill seeding treatment. Seeds or pellets were hand broadcasted evenly across plots that received a broadcast seeding treatment. Vegetation cover and density by species were measured during the summers of 2016 and 2017 in each Year 2 treatment.

Year 1 and 2 results were summarized and are presented and discussed below.

Results

Differences occurred in forage kochia establishment success between Year 1 (seeded in fall 2014 and late winter 2015) and Year 2 (seeded in fall 2015 and later winter 2016) in the study. Overall establishment success associated with Year 2 seeding treatments was much lower than that associated with Year 1 seeding treatments (Figures 1-4). This disparity may have been related to differences in precipitation among the two establishment years (Table 1). While the sites experienced similar crop year (October – September) precipitation during 2014-2015 and 2015-2016 (Table 1), the additional half inch of precipitation received in Year 1 occurred largely during a potentially critical time for kochia establishment in late spring and summer.

Results from the Year 1 seeding suggested planting during the winter was a more effective time for establishing forage kochia than fall planting, regardless of whether stored (i.e., year-old) or freshly harvested seed was used (Figure 1). Site 1 demonstrated a similar result for the Year 2 seeding when kochia was drill seeded (Figure 2-A). It appears that fall-seeded forage kochia might germinate and emerge shortly after planting and subsequently suffer high winter mortality, whereas emergence of seed sown in the winter is likely delayed until a more favorable time during the spring. Site 2 experienced a longer period of snow cover after the Year 2 fall planting which may have mitigated winter mortality risk leading to similar establishment densities among fall and winter plantings (Figure 2-B).

Shallow drill seeding conducted in the winter tended to produce better initial establishment than broadcasting seed at both Sites 1 and 2 in the Year 1 seeding and at Site 1 in the Year 2 seeding (Figures 1-4). Results from the Year 1 seeding also indicated drilling bare seed outperformed seed sown during the winter in pellets (Figure 1), enhancements designed to improve seed germination and emergence, suggesting seed germination and emergence may not be major limiting factors for forage kochia establishment when seeding during the winter months. Similarly, seeds that had been stored for a year and then treated with a hydrophobic coating, an enhancement designed to delay germination of fall-planted seed until spring, performed poorly compared to similar untreated seed (i.e., year-old seed) planted in the winter (Figures 1 and 3). Again, results of this study indicate winter seeding is generally superior to fall seeding which suggests delaying germination of fall-seeded forage kochia until late winter or spring should improve establishment performance of forage kochia. Therefore, we suspect the amount and/or formulation of the hydrophobic coating tested in this study needs adjustment, and deserves further investigation.

Conclusions

In conclusion, results from this experiment indicate that shallow drill seeding forage kochia during late winter (February) will generally increase its initial establishment success over broadcast seeding in winter and broadcast or drill seeding during the fall. In addition, we did not observe a marked difference in the performance of year-old seed that had been properly stored compared to freshly harvested seed when both were seeded during the winter. We suspect struggles with using year-old seed are likely related more to the timing of seeding (fall vs winter) than seed age, whereby seed available for fall planting has, by definition, been stored for at least one year. Our results suggest this seed will perform satisfactorily if properly stored and planted in the winter. Lastly, seed enhancement treatments designed to improve germination and emergence did not improve establishment of forage kochia over shallow drill seeding in the winter, suggesting seed germination and emergence may not be limiting factors for establishment if appropriate planting timing and techniques are employed.

Acknowledgments

This research study was financially supported by the Oregon Beef Council.

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Table 1. Crop year and monthly precipitation totals for Sites 1 and 2 during establishment Years 1 and 2 of the study.

	Site 1		**Site 2**	
	Year 1	Year 2	Year 1	Year 2
	(inches)		(inches)	
October	0.66	1.15	0.69	1.23
November	1.32	0.97	1.33	1.11
December	1.81	2.22	2.05	2.98
January	0.32	1.01	0.51	1.25
February	0.96	0.29	1.21	0.44
March	0.57	0.88	0.78	1.28
April	0.67	0.45	0.64	0.48
May	2.38	1.88	2.37	1.35
June	0.02	0.39	0.12	0.23
July	0.87	0.32	0.87	0.36
August	0.05	0.02	0.06	0.00
September	0.92	0.3	0.5	0.12
Total Crop Year	10.55	9.88	11.13	10.83

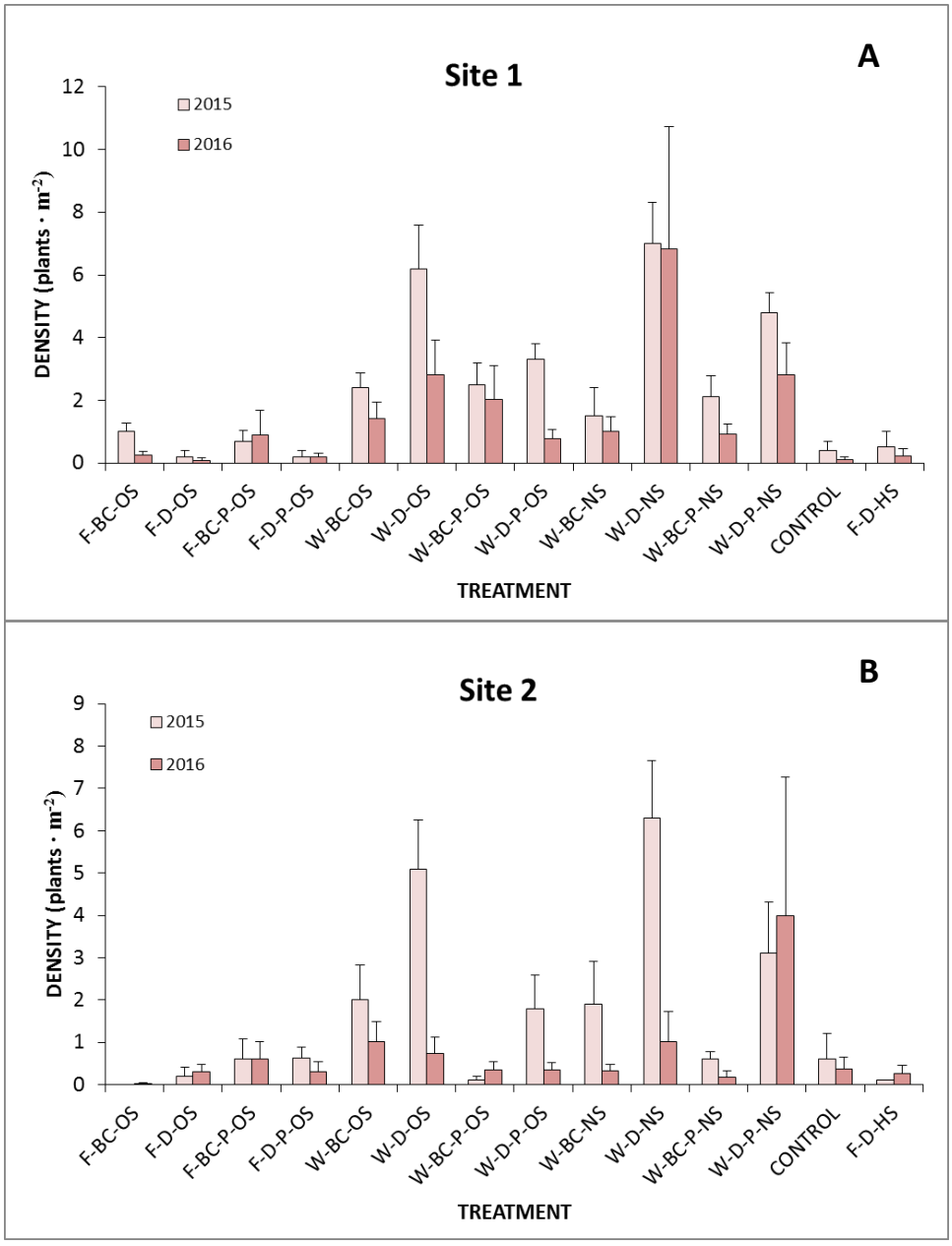

Figure 1. Density (mean + SE) of forage kochia the two growing seasons after Year 1 seeding treatments were applied. F-BC-OS: Fall broadcast of year-old seed; F-D-OS: Fall drill of year-old seed; F-BC-P-OS: Fall broadcast of pillows made with year-old seed; F-D-P-OS: Fall drill of pellets made with year-old seed; W-BC-OS: Winter broadcast of year-old seed; W-D-OS: Winter drill of year-old seed; W-BC-P-OS: Winter broadcast of pillows made with year-old seed; W-D-P-OS: Winter drill of pellets made with year-old seed; W-BC-NS: Winter broadcast of new seed; W-D-NS: Winter drill of new seed; W-BC-P-NS: Winter broadcast of pillows made with new seed; W-D-P-NS: Winter drill of pellets made with new seed; CONTROL: Not seeded; and F-D-HS: Fall drill of old seed with hydrophobic coating.

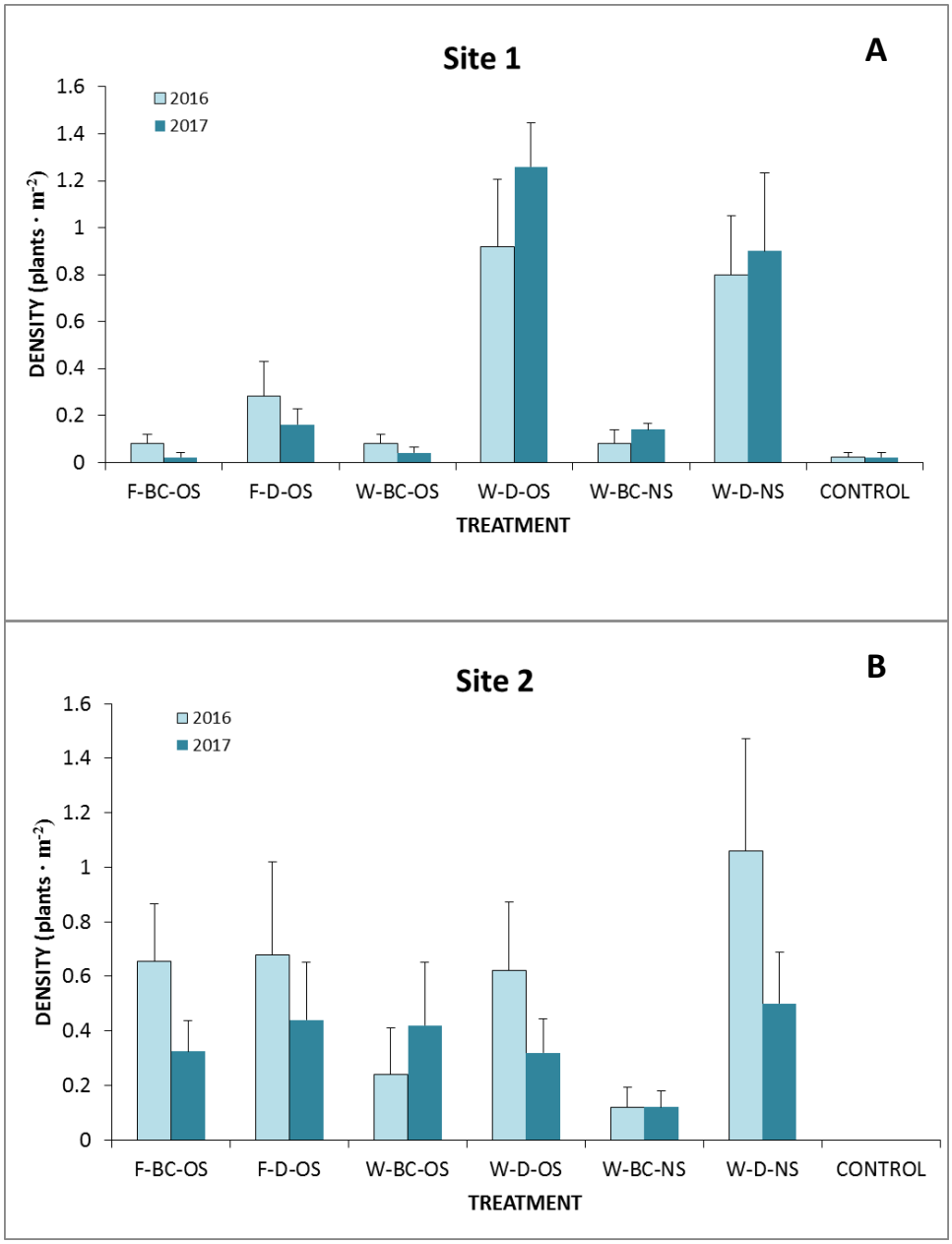

Figure 2. Density (mean + SE) of forage kochia the two growing seasons after Year 2 seeding treatments were applied. F-BC-OS: Fall broadcast of year-old seed; F-D-OS: Fall drill of year-old seed; W-BC-OS: Winter broadcast of year-old seed; W-D-OS: Winter drill of year-old seed; W-BC-NS: Winter broadcast of new seed; W-D-NS: Winter drill of new seed; CONTROL: Not seeded.

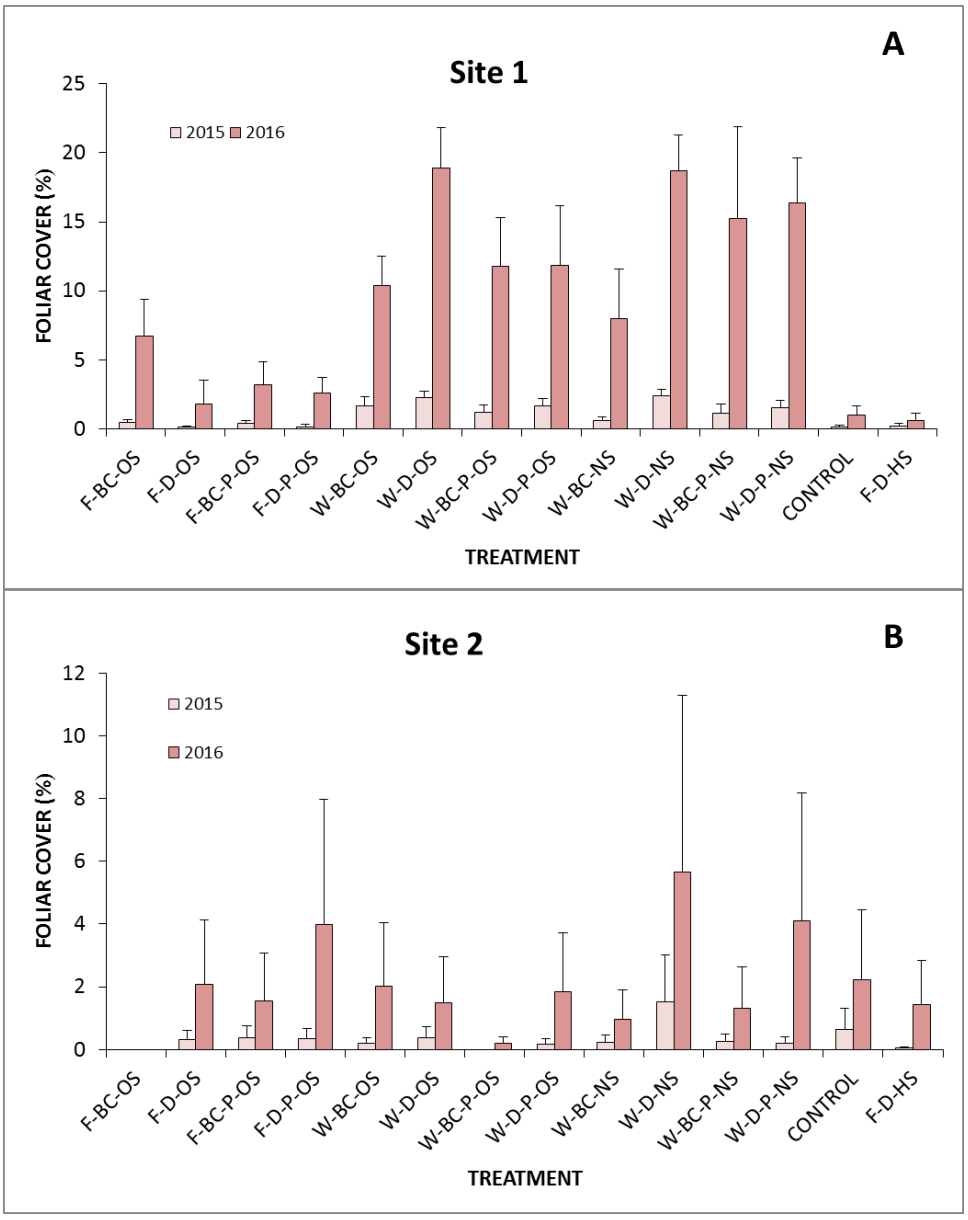

Figure 3. Foliar cover (mean + SE) of forage kochia the two growing seasons after Year 1 seeding treatments were applied. F-BC-OS: Fall broadcast of year-old seed; F-D-OS: Fall drill of year-old seed; F-BC-P-OS: Fall broadcast of pillows made with year-old seed; F-D-P-OS: Fall drill of pellets made with year-old seed; W-BC-OS: Winter broadcast of year-old seed; W-D-OS: Winter drill of year-old seed; W-BC-P-OS: Winter broadcast of pillows made with year-old seed; W-D-P-OS: Winter drill of pellets made with year-old seed; W-BC-NS: Winter broadcast of new seed; W-D-NS: Winter drill of new seed; W-BC-P-NS: Winter broadcast of pillows made with new seed; W-D-P-NS: Winter drill of pellets made with new seed; CONTROL: Not seeded; and F-D-HS: Fall drill of old seed with hydrophobic coating.

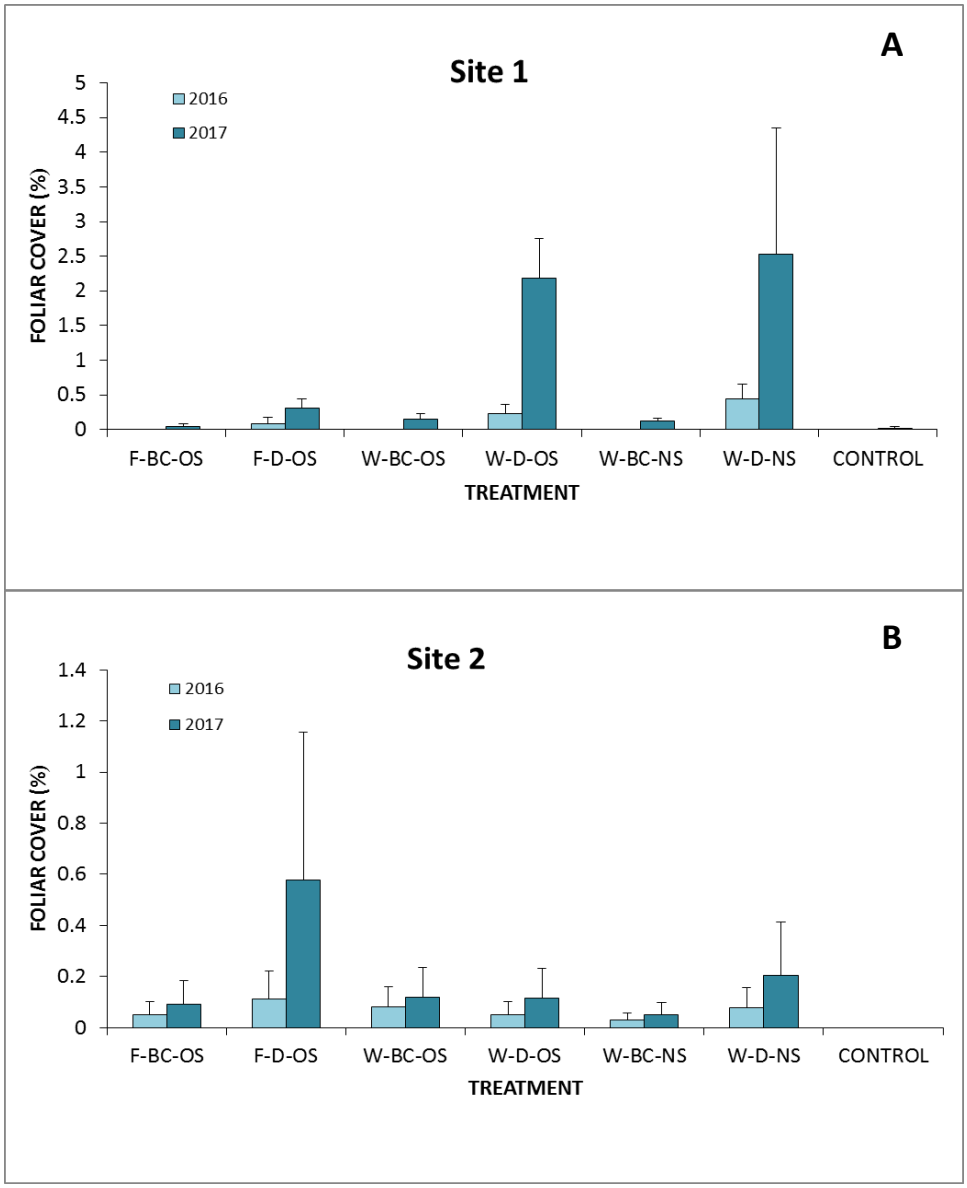

Figure 4. Foliar cover (mean + SE) of forage kochia the two growing seasons after Year 2 seeding treatments were applied. F-BC-OS: Fall broadcast of year-old seed; F-D-OS: Fall drill of year-old seed; W-BC-OS: Winter broadcast of year-old seed; W-D-OS: Winter drill of year-old seed; W-BC-NS: Winter broadcast of new seed; W-D-NS: Winter drill of new seed; CONTROL: Not seeded.

BEEF158

Oregon State University

Oregon Beef Council Report

Beef Cattle Sciences

Preventing juniper reestablishment into sagebrush communities: Improving the watershed function [1]

Carlos G. Ochoa[2]

Synopsis

Ongoing research related to juniper reinvasion and its potential implications for the overall watershed function.

Summary

The present report shows preliminary results from ongoing field data collection and analyses of multiple vegetation and hydrology-related variables. Overall, it has been observed that the number of juniper saplings (46 tree/acre) in the treated watershed is nearly the same than the number of mature trees observed in the untreated watershed. Juniper saplings in the treated watershed range from 2 to 15 years old. Mature juniper stands can intercept up to 46% of total precipitation. Greater springflow and streamflow levels were observed in the treated watershed. Seasonal aquifer recharge in the entire watershed-riparian system ranges from 2.5 to 4.5 ac-ft/ac, and it is heavily dependent on winter precipitation mainly in the form of snow.

Introduction

Striking landscape changes attributed to high levels of encroachment into sagebrush-steppe (Miller et al., 2001; Chambers, 2001) and grassland ecosystems (Coppedge et al., 2004; Taylor et al., 2007) have raised considerable concerns about the negative impacts of juniper expansion on multiple ecosystem functions and services provided. Juniper encroachment into these rangeland ecosystems can limit the growth of shrubs, grasses, and forbs, by outcompeting them for light, soil moisture, and soil nutrients (Vaitkus and Eddleman, 1987; McPherson and Wright, 1990; Gottfried et al., 2000), reduce biodiversity (Tausch and West, 1995; Miller et al., 2000; Bates et al., 2002), alter soil nutrient cycling (Bates et al., 2002), and modify hydrologic processes such as evapotranspiration and soil moisture (Wilcox, 1994; Petersen et al., 2008; Mollnau et al., 2014). It is increasingly recognized that juniper expansion effects on groundwater recharge must be better understood and that comprehensive resource management requires evaluation and integration of surface water and groundwater components. Surface water and groundwater cannot be seen as two isolated entities, there are multiple interactions between these two components that occur throughout the landscape. Surface water and groundwater connections throughout the landscape can determine multiple biophysical relationships that are critical for the productivity of a given site.

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Materials and Methods

The objectives of this ongoing research effort are: 1) To characterize the progression of juniper re-occupation of sagebrush communities ten-years after tree removal, and; 2) To evaluate potential impacts of preventing juniper re-occupation on sagebrush steppe vegetation and hydrologic dynamics. The project is being conducted in the Camp Creek-Paired Watershed Study (CCPWS) site, 17 miles northeast of Brothers, OR. The study area comprises one 290-acre watershed (Mays WS), one 237-acre watershed (Jensen WS), and a 50-acre section (Riparian Valley) of the West Fork Camp Creek. Dominant overstory vegetation in Jensen WS is western juniper (Juniperus occidentalis). Dominant overstory vegetation in Mays WS is big sagebrush (Artemisia tridentata), this was after approximately 90% of the juniper was removed in 2005 (Deboodt 2008). The Riparian Valley site is largely a grassland (various spp.) area within two low dams and it is surrounded by sagebrush and western juniper vegetation. In 2005, the two watersheds were instrumented to monitor multiple hydrologic variables including precipitation, soil moisture, runoff, and groundwater. Since October 2014, new instrumentation to measure selected variables (i.e., soil moisture, rainfall, and groundwater) has been added to expand the monitoring network in the watersheds and to include the Riparian Valley site (Figure 1).

Our ongoing field data collection and analyses efforts use a combination of traditional rangeland monitoring techniques coupled with automated recordings of hydrologic variables (e.g., precipitation, soil moisture, and groundwater levels) and aerial imagery to assess the overall effects of juniper reestablishment in the watershed function. We are documenting juniper canopy cover and density in both the treated watershed and the untreated watershed. We are using the belt transect method to determine juniper sapling stem density and canopy cover in the treated watershed. We are using a combination of satellite imagery and on-the-ground measurements to document mature tree canopy cover and density in two plots in the untreated watershed. Various juniper saplings have been cut to extract cross-sections to determine tree age using ring count techniques. We are analyzing the relationships between surface water and groundwater sources in both watersheds and in the riparian valley using graphic and statistical analyses.

Results

Preliminary research results indicate there is a density of 46 trees per acre in the treated watershed, with tree age ranging from 2 to 12 years old. Total canopy cover occupancy is <2%. Low altitude aerial imagery has been used in combination with on-the-ground measurements to provide a better visual representation of juniper reestablishment throughout the landscape (Figure 2).

We delineated two experimental plots of approximately 0.5 acre each to account for the number of juniper trees in one upstream and one downstream location in the untreated watershed. Canopy cover estimates of mature trees were 29% at a downstream location and 26% at an upstream location sampled in the untreated watershed (Figure 3). Juniper-canopy cover interception at these two sites accounted for 46% (downstream site) and 36% (upstream site) of total annual precipitation.

The analysis of various hydrologic variables indicate the seasonality and general trend of surface water and groundwater relationships across the study site. For example, study results showed there is a strong soil moisture response to winter-season precipitation inputs in both watersheds and in the riparian valley. Figure 4 illustrates the seasonal pattern of daily-averaged soil moisture fluctuations collected from monitoring stations installed at upper and lower locations in each watershed. Overall, greater levels of soil moisture content were observed in the top 20 inches (0.5 m) soil profile. A delayed soil moisture response in the deepest sensor at 32 inches (0.8 m) was observed at all four locations during the drier 2013-2014 winter season (Figure 4).

Springflow data analysis showed spring discharge in both watersheds followed a seasonal pattern corresponding to regional precipitation dynamics (dry summers, wet winters). In general, springflow rates began increasing in late winter, peaked in mid-spring, and then followed a steady decline until reaching baseline levels in autumn

(Figure 5). It was always the case that the treated watershed had higher springflow rates than the untreated watershed. However, while flow rates in the untreated watershed remained relatively flat throughout the entire period of record (2005-2017), springflow rates in the treated watershed had shown an upward positive trend after juniper removal that happened during 2005-2006. It is noteworthy to mention that no data were collected in years 2014 and 2015; however, the average precipitation conditions observed during those two years indicated that springflow rate trends may have remained the same for both watersheds.

Aquifer recharge estimates for the two watersheds and riparian valley were calculated using automated data collected over the past three and a half years (2014-2017). Highly variable recharge estimates were obtained from the three different locations. However, the two watersheds followed a similar aquifer recharge pattern, which is consistent with groundwater level rise dynamics observed in both locations. The treated watershed had greater aquifer recharge estimates in years 2015 and 2017 when compared to the untreated watershed. The greatest aquifer recharge estimate of 784 mm in the riparian valley corresponds to the observed replenishment of the shallow system following a drier 2015 year. (Table 1).

Conclusions

Results from this ongoing study indicate that juniper density is nearly the same in both the treated watershed and the untreated watershed. As expected, mature tree canopy cover levels in the untreated watershed are significantly higher than sapling canopy cover in the treated watershed. The similarity in tree density for both watersheds suggests that a secondary treatment is necessary to prevent juniper growth to a mature stage that can decrease site productivity in the treated watershed. The ongoing analyses of hydrologic variables indicate clear seasonal patterns of soil moisture and aquifer recharge in response to winter precipitation inputs. The long-term analysis of springflow data shows there is an upward trend in spring discharge levels in the treated watershed. Spring discharge levels have remained relatively the same in the untreated watershed.

Acknowledgments

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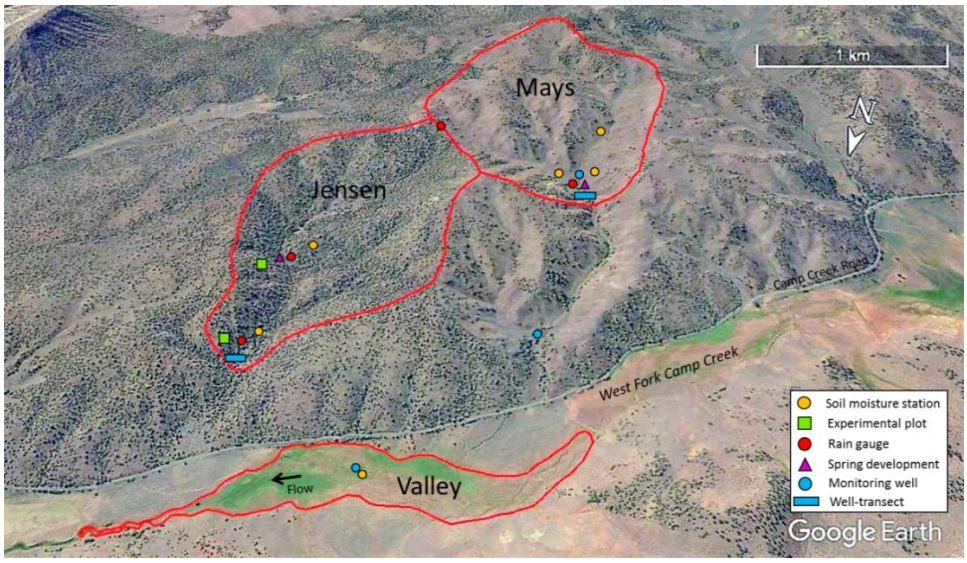

Figure 1. Map of the study site showing the Mays WS (a), the Jensen WS (b), and the Riparian Valley area (c), indicating the location of different monitoring instrumentation used in this study.

Figure 2. Low altitude aerial image illustrating the belt transect method and some of the juniper saplings commonly found in the treated watershed.

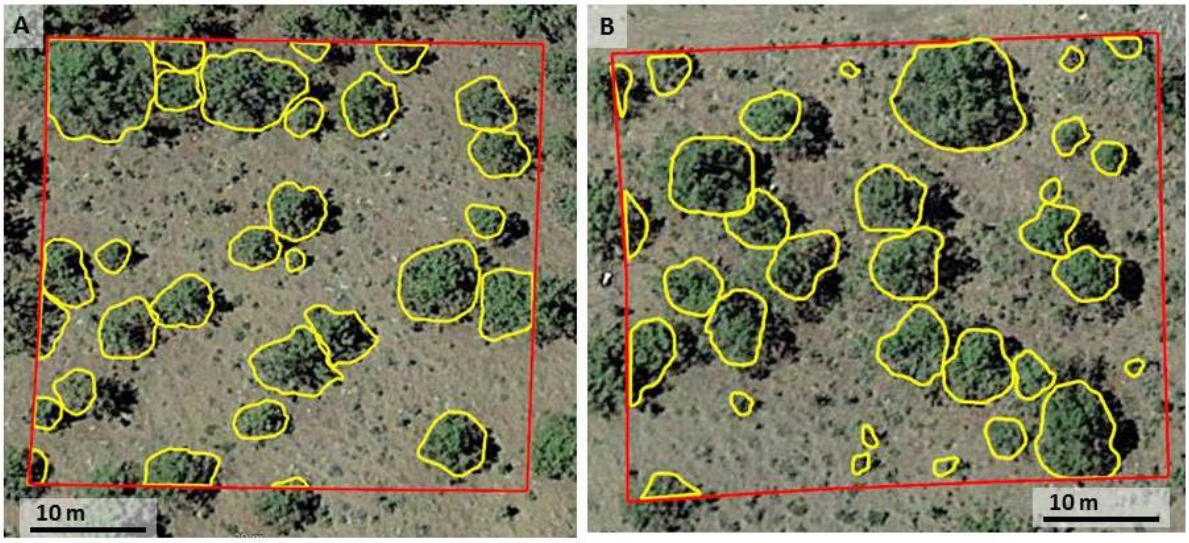

Figure 3. Satellite images illustrating juniper trees (outlined in yellow) used in estimating total canopy cover for the downstream (A) and upstream (B) experimental plots.

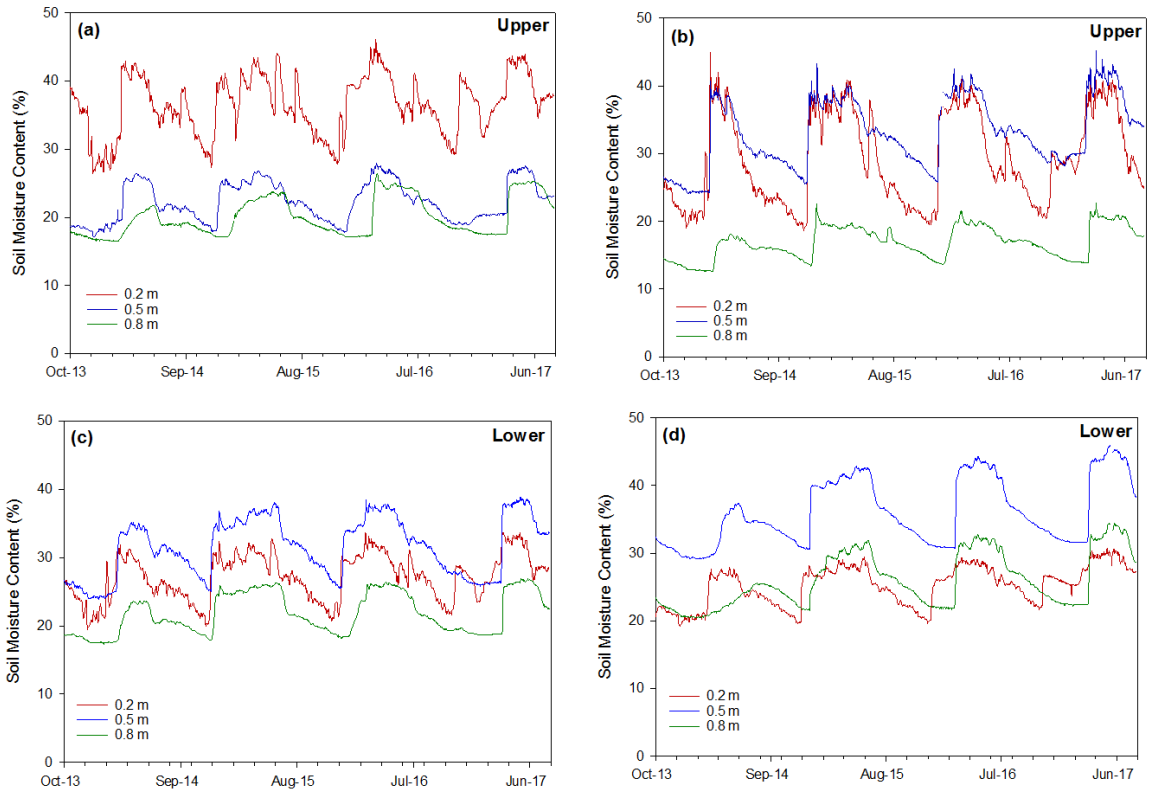

Figure 4. Soil moisture content fluctuations at different soil depths at upper and lower locations in both Mays WS (a, c) and Jensen WS (b, d) watersheds from 1 October 2013 through 27 July 2017.

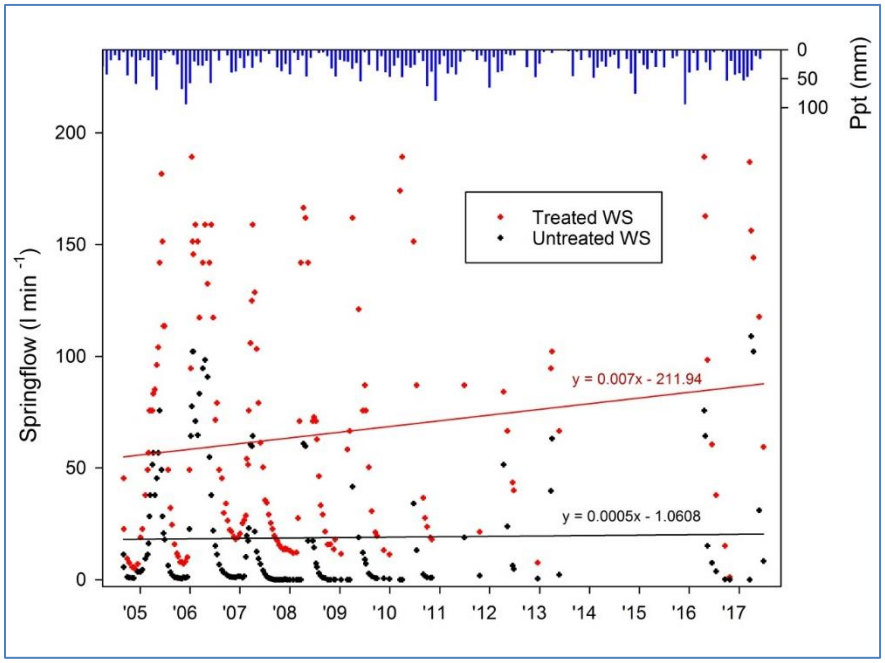

Figure 5. Springflow rate estimates for selected dates and monthly precipitation (Ppt) totals obtained for both watersheds (WS) from September 2004 through June 2017.

Table 1. Aquifer recharge estimates based on the Water Table Fluctuation Method for the two watersheds and the riparian valley.

Water Year	Untreated (mm)	Treated (mm)	Riparian (mm)
2015	693	1158	678
2016	1326	1173	784
2017	1410	1445	651

BEEF159

Oregon State University

Oregon Beef Council Report

Beef Cattle Sciences

Annual Warm Season Grasses for Forages: Enhancement of Quality and Production of Forages under Limited and Competing Water Resources in Eastern Oregon[1]

Guojie Wang[2]

Synopsis

Annual warm season grass species could be used as forages in eastern Oregon with production level of more than 5 tons/acre under fully irrigation and more than 3 tons/acre under limited irrigation or dryland situations.

Summary

Five annual warm season grass species were tested in the field under four irrigation treatments in eastern Oregon. Irrigation from May 1 to September 15 is no different from irrigation from May 1 to August 1. Pearl millet and foxtail millet produced the most forage under irrigation from May 1 to August 1 with an average over 5 tons/acre. Sorghum-sudan grass produced the most forage under irrigation from May 1 to June 15 and no irrigation situations with an average over 3 tons/acre. Annual warm season grasses can be used as forage species in eastern Oregon under drought situations.

Introduction

The climate in Eastern Oregon is close to Mediterranean type, with wet and mild winters, dry and hot summers. The precipitation in June, July, August, and September is scarce. Therefore, the dominant perennial forage species such as blue bunch wheatgrass and Idaho fescue go to their dormancy without irrigation in summer months. However, the forage demand in summer months is comparable to spring and fall, if it is not more. We could call the forage gap in summer months as "summer depression" and unfortunately, this situation will be worse and worse with the climate change introduced summer precipitation decreases in Eastern Oregon. Furthermore, eastern Oregon is not only facing limited water resources, but also competing with other water demanding sectors, such as wildlife habitat, creek ecosystems, and municipal demand.

In order to overcome the summer depression in forage production, forage and beef producers in eastern Oregon need search a specific forage production system that conserve water use with higher water use efficiency, and in the same time produce comparable or higher quantity and quality forages. One direction of this search could be annual

1. This document is part of the Oregon State University – 2017 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.\
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warm season grasses such as millets, sorghums, sudangrasses, and sorghum-sudan hybrids (McCartney et al., 2009). Due to their warm season growing pattern, annual warn season grasses can grow into hot summers further than cool season grasses. They are also drought tolerant species compared with other common annual forages. Furthermore, annual forages production system can present flexibility to beef production sector to fill in any forage gaps in the management. However, annual warm season forages are overlooked in forage and beef production sectors due to misunderstanding of their forage quality, shortage of information, and precise management protocols.

New forage cultivars of annual warm season grasses were developed in last decade such as brown mid-rib (BMR) sorghums and its hybrids to improve their forage quality (McCollum, et al., 2003). Annual warm season grass forages quality also depends on harvest timing and soil fertility. Therefore, we believe that annual warm season grasses for forages deserve a detailed screening and evaluation, especially with limited water availability situation in eastern Oregon. Therefore, the objective of this study is to study annual warm season grasses production and quality under different irrigation treatments. The specific objectives are: 1) Screen annual warm season grass forage species and cultivars for forage species and cultivars selection; 2) Compare annual warm season grasses forage production and quality under different irrigation treatment in eastern Oregon.

Materials and Methods

The field study was carried out at Eastern Oregon Agricultural Research Center at Union Station in 2017. The field plots were laid out as split plot design with four replications. Irrigation treatment was the whole plot, different annual warn season grass species and cultivars were the subplots. Irrigation treatments included 1) W1—irrigation from May 1 to September 15; 2) W2—irrigation from May 1 to August 1; 3) W3—irrigation from May 1 to June 15; and 4) W4—no irrigation under dryland situation. Approximately two inches of water was applied to the testing plots area each week based on irrigation treatment lay out. Irrigation treatments matches water rights in this region as well as matches major forage species growth pattern. Annual warm season grass species included German millet, pearl millet, proso millet, teff, and sorghum-sudan hybrids (Table 1).

Soil was tested for soil fertility and fertilization recommendations. Urea (46-0-0), ammonium phosphate (11-52-0), and potash (0-0-60) were applied as needed fertilizers according to soil test results before seeding. The germinated annual weeds and the regrowth of perennial vegetation in the plots area were controlled by glyphosate application. The selected annual warm season grasses were seeded on June 14, 2017 based on the recommended seeding rates (Table 1). Annual warm season grasses germination requires soil temperature above 55 F, so seeding them late in spring is necessary to have a good start of germination and growth. Post emergence herbicides were applied to control broadleaf weeds.

The germination and growth of annual warm season grass species and cultivars was observed and recorded by frequent field visits and monitoring. The accepted and standard growing stage measures and methods was used for annual warm season grass phenological development monitoring. The production data was collected by harvesting each plot based on each species and cultivar maturity stage. The harvesting stage was heading stage for this study, which is between booting and anthesis stages being recommended from different researchers. Proso millet and teff were harvested on August 17 and the other three species were harvested on September 6. The harvested material was oven dried, and converted into pounds per acre on a dry matter basis. The oven dried plant material was grounded and ready for forage quality lab analysis.

Results

Irrigation treatment, annual warm season grass species, and their interactions had significant effects on forage production. Averaged over five species, forage production under irrigation from May 1 to September 15 and from May 1 to August 1 was comparable, which was significantly higher than

irrigation from May 1 to June 15 and no irrigation (Figure 1). Average over four irrigation treatments, forage production of sorghum-sudan, foxtail millet, and pearl millet was comparable, which was significantly higher than proso millet and teff (Figure 2)

Foxtail millet and pearl millet produced more than 6 tons/acre under irrigation from May 1 to September 15 and from May 1 to August 15 (Table 2). Sorghum-sudan hybrids produced the highest amount forage under irrigation from May 1 to June 15 and no irrigation (Table 2). Without irrigation or without late season irrigation, teff could not grow well in eastern Oregon.

Discussion

The five warn season grass species produced comparable amount of forage under irrigation from May 1 to September 15 and from May 1 to August 1. The similarity between those two irrigation treatments are related to species growth curves and growing season conditions. In the middle of August, proso millet, foxtail millet, and teff are ready to be harvested based on their maturity stage. Therefore, extra irrigation from August 1 to September 15 had minimum effects on these three species production. On the other hand, the first killing frost of 2017 at Union happened on September 15. The short growing season in eastern Oregon does not permit later maturing species such as pearl millet and sorghum-sudan grass to grow into their potential. The soil under this study is silt loam, which has high level of organic matter content and water holding capacity. Fully irrigation on August 1 coupling with low air temperature probably is enough for these five warm season grasses to grow before the first killing frost.

Foxtail millet and pearl millet produced the most forage under full irrigation or near full irrigation. Meanwhile, sorghum-sudan grass produced the most forage under limit early season irrigation and no irrigation situations. The species different response to irrigation treatment would be related to their drought tolerance mechanisms and their growth curves. Pearl millet matured for forage harvesting around the first week of September. It matches well with eastern Oregon weather conditions. Sorghum-sudan grass matured for forage harvest two weeks later. With full irrigation, pearl millet can reach its growth potential. However, sorghum-sudan cannot reach its growth potential due to the short growing season. Under limited irrigation, the maturity of all species was delayed. Sorghum-sudan seems to way to cope with drought situations, as demonstrated by other researchers, such as deep rooting depth and tillering capacities.

We seed annual warm season grasses in the middle of June due to the consideration of soil temperature requirement for seed germination and seedling growth. If we can seed these annual warm season grasses two weeks earlier, the production and reaction with different irrigation treatments would be significant different. We will test different seeding date effects on annual warm season grasses performance in the future.

This is only a one-year study with limited references to year-to-year variations in the climatic conditions in eastern Oregon. Multi-year and multi-site study would be preferred to enlarge the results applicable areas. All the forage quality data will be presented as soon as the lab analysis data available.

Acknowledgments

This research study is financially supported by the Oregon Beef Council.

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Table1. Annual warm season grasses seeded in spring 2017 at Eastern Oregon Agricultural Research Center, Union

Species	Cultivar	Seeding Rate (lb/acre)
Proso Millet	White	30
Foxtail Millet	Golden German	20
Pearl Millet	MS2500	20
Teff	Tiffany	10
Sorghum-Sudan Hybrid	Cow Conditioner BMR	40

Table 2. Forage production (lbs/acre) of annual warm season grasses seeded in spring 2017 at Eastern Oregon Agricultural Research Center, Union. W1: irrigation from May 1 to September 15; W2: irrigation from May 1 to August 1; W3: irrigation from May 1 to June 15; W4: no irrigation under dryland situation.

Species	Irrigation Treatment			
	W1	W2	W3	W4
Foxtail millet	12563a	12488a	6280b	2551c
Pearl millet	12337a	12092a	5429c	3530b
Proso millet	8385c	8468c	6073b	2542c
Sorghum-sudan	10719b	10756b	7946a	6433a
Teff	5328d	5187d	154d	0d

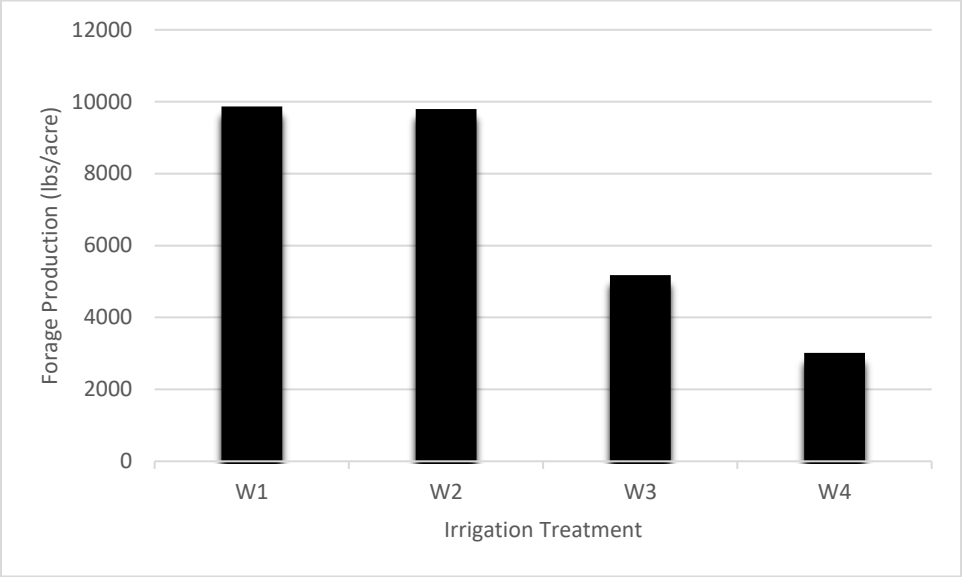

Figure 1. Average forage production (lbs/acre) of five annual warm season grasses seeded in spring 2017 under four irrigation treatments at Eastern Oregon Agricultural Research Center, Union. W1: irrigation from May 1 to September 15; W2: irrigation from May 1 to August 1; W3: irrigation from May 1 to June 15; W4: no irrigation under dryland situation.

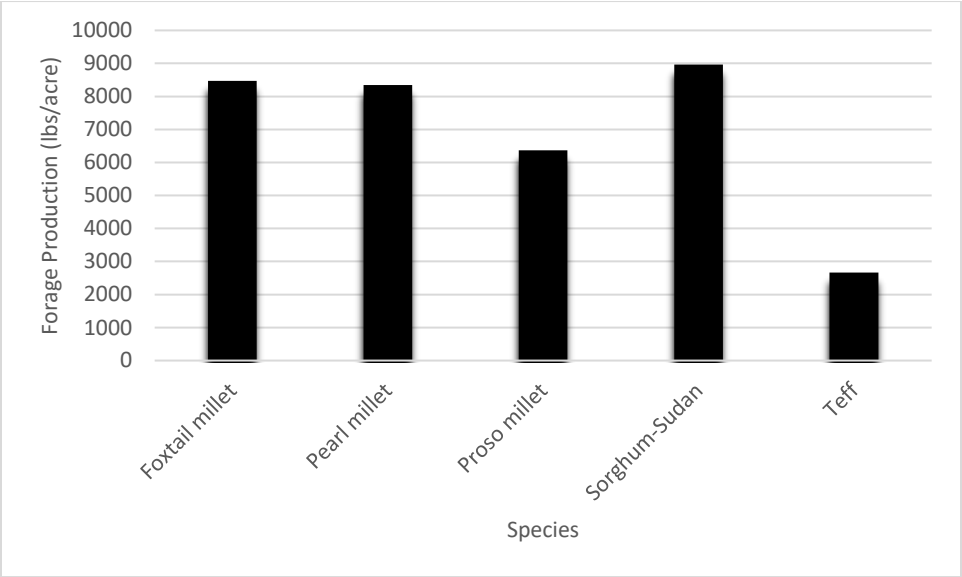

Figure 2. Average forage production (lbs/acre) over four irrigation treatments of five annual warm season grasses seeded in spring 2017 at Eastern Oregon Agricultural Research Center, Union.

Oregon State University

BEEF160

Oregon Beef Council Report

Beef Cattle Sciences

Greater sage-grouse response to landscape level juniper removal[1]

Christian Hagen[2]

Synopsis

Use Landscape level removal of juniper improved sage-grouse nest survival and annual adult female survival in the treatment area (area with juniper removal) relative to the control (area without juniper removal).

Summary

Conifer encroachment of sagebrush ecosystems is considered a key a threat to sage-grouse (*Centrocercus urophasianus*) populations in the Great Basin. Management agencies are actively removing encroaching conifers such as juniper (*Juniperus* spp.) to benefit sage-grouse. However, little is known about how sage-grouse populations will respond to these landscape level management actions. A long-term project was established in Lake County, Oregon using a before-after-control-impact framework to assess the effects of juniper removal. Female sage-grouse were marked with necklace VHF transmitters or GPS backpack transmitters in a treatment area (area with juniper removal) and control area (area without juniper removal) and monitored throughout the year from 2015–2017. This work was in addition to an existing data set collected in the project area from 2011–2014. Telemetry data was analyzed in Program MARK to estimate nest, chick, breeding season, and annual survival in the treatment and control areas. The results of this analysis indicated generally positive effects of juniper removal on nest and annual survival. The short term (3 years post-removal) effects of juniper removal were positive on breeding season survival, but this pattern was not as evident during the later phase of the project. The effects on chick survival were unclear because they may have been heavily skewed due to an extreme difference in chick survival in 2011. The magnitude of this difference may not represent the true difference in chick survival rates between the treatment and control areas in 2011.

Introduction

Conifers such as western juniper have expanded into sagebrush ecosystems in the Great Basin largely due to anthropogenic changes in wildfire return intervals (Miller and Rose, 1999). These range expansions are considered a primary threat to greater sage-grouse (Centrocercus urophasianus; hereafter sage-grouse; Baruch-Mordo et al., 2013), a sagebrush obligate that has undergone an approximately 50% range contraction (Schroeder et al., 2004). Sage-grouse are known to avoid junipers at canopy cover levels as low as 3% (Severson et al., 2016). Increasing juniper cover in sagebrush ecosystems may negatively affect sage-grouse due to increased predation as a result of greater hiding

1. This document is part of the Oregon State University – 2017 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.\
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cover for mammalian predators and more numerous perches for avian predators. However, little is known about the impacts of broad-scale removal of encroaching conifers. A treatment area with juniper removal and a control area without any juniper removal were established as a long-term study site in Lake County, Oregon to assess the impact of landscape level juniper removal on sage-grouse (Figure 1). Juniper removal began in the treatment area in 2012 and the majority of removals were completed by the end of 2014. To date, juniper have been removed across approximately 13,000 ha of the treatment area. Data was collected from 2015–2017 to build upon the existing, long-term database for the project from 2011 (pre-treatment year)–2014. Using a before-after-control-impact (BACI) framework, we assess adult female survival, nest survival, and chick survival to test the hypothesis that removal of juniper will improve these vital rates at the long-term study site in Oregon.

Materials and Methods

Female sage-grouse were captured from 2015–2017 in the treatment and control areas with the goal of maintaining a sample size of approximately 40 in each area at the start of the breeding season. Captured sage-grouse were marked with VHF necklace transmitters or GPS backpack transmitters. Individuals marked with VHF transmitters were located twice weekly by technicians April–July and once monthly August–March by plane. Locations for individuals marked with GPS transmitters were recorded 4–5 times per day year-round.

All telemetry data was analyzed using Program MARK. Adult female annual (April–March) survival and breeding season (April–July) survival were estimated using known fate models. Nest survival (27 days) was analyzed using nest survival models. Chick survival to 54 days was analyzed using the Lukacs young survival from marked adults model. It was assumed that the pre-treatment (2011) difference in vital rate estimates between the treatment and control areas (treatment-control) was the "inherent difference" between these areas prior to juniper removal. To account for this, the differences in model-averaged derived estimates were standardized by adjusting the observed difference in the 2011 vital rate estimates to zero and carrying that same correction forward through subsequent years.

Results

We captured and marked 121 female sage-grouse and monitored 153 nests and 78 broods (Table 1). Nest survival standardized differences were positive with the exception of 2015, indicating a generally positive effect of juniper treatments on 27-day nest survival (Figure 2; Table 2). A positive trend in nest survival rates was particularly apparent during the first three years post-removal (2012–2014; Figure 2). Standardized differences in chick survival to 54 days were negative post removal (Table 2). However, the extreme difference in survival rates in 2011 (Table 2), which determined the adjustment of subsequent years, may be an outlier and inserts substantial uncertainty into these estimates. If adjustments were made based on the 2012 estimates, then all differences post-2012 would be positive indicating a positive effect of juniper removal on chick survival. Similar to nest survival, breeding season survival standardized differences had a positive trend from 2012–2104, but this pattern was not apparent during the current phase of the project (Table 2). Standardized differences in annual survival had a positive trend over the course of the entire project (Figure 3; Table 2). By 2016, there was a 7.5% increase in annual survival in the treatment relative to the control.

Conclusions

The preliminary results of this project indicate that landscape level removal of conifers such as juniper can be an effective tool for increasing sage-grouse vital rates such as nest and annual survival. These vital rates are significant determinants of sage-grouse population growth rates (Taylor et al., 2012; Dahlgren et al., 2016). Further demographic analyses will incorporate vital estimates from telemetry and lek counts into integrated population models for a more robust understanding of the efficacy of juniper removal as a tool to maintain or

increase sage-grouse abundance. Additionally, the impacts of juniper on sage-grouse seasonal movements from nesting to late brood/summer habitat as well as the dynamics of sage-grouse habitat selection in relation to juniper removal will be investigated.

Acknowledgments

This research study was financially supported by the Oregon Beef Council, Bureau of Land Management, USDA's Natural Resources Conservation Service- Conservation Effects Assessment Project, and Oregon Department of Fish & Wildlife.

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Taylor et al. 2012. J. Wildl. Man. 76:336–347.

Table 1. Newly marked female sage-grouse, monitored nests, and monitored broods in the juniper removal treatment and control areas, 2015–2017, Lake County, Oregon.

	2015		2016		2017	
	Treatment	Control	Treatment	Control	Treatment	Control
Hens Marked	17	20	23	14	13	34
Nests Monitored	27	27	27	24	20	28
Broods Monitored	17	16	13	12	8	12

Oregon State University

Oregon Beef Council Report

Beef Cattle Sciences

Progress Reports – Rangeland Ecology and Management [1]

Developing Conservation Measures to Restore and Rehabilitate Rangelands on Degraded Sage-Grouse Habitat in Southeastern Oregon

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Project Objectives: The objectives of this experiment were to: 1) implement one mechanical and one fire treatment to restore and/or rehabilitate degraded sage-grouse habitat on four ranches with deeded property enrolled in the Greater Sage-Grouse Candidate Conservation Agreement with Assurances (CCAA); 2) apply native and introduced seeding treatments to five experimental plots on four cow-calf ranches; 3) measure and evaluate plant community responses two years (April through November) after using mechanical, chemical, and fire treatments, as well as native and introduced fall seedings on Ecological State C, low elevation sagebrush rangelands.

Project Start Date: Fall of 2017
Project Completion Date: Fall of 2020

Project status: In 2016, *Ecological State C* sites were selected on four cow-calf operations in Malheur and Harney counties whose managers were influential in enrolling land in CCAAs. These sites were selected due to decadent sagebrush and Sandberg bluegrass and/or annual grasses dominance. Sagebrush cover on these sites is greater than 10% and is capable of providing seasonal sage-grouse habitat. However, these sites lack resiliency after disturbances like wildfire because they are largely devoid of desired, deep-rooted perennial understory grasses and forbs and occur in warm, dry areas of the sagebrush ecosystem that are most prone to invasion by exotic annual grasses like cheatgrass and medusahead. In Fall 2016, exclosures were established and baseline data were recorded for cover and density of grass, forb, and shrubs. The following fire, mechanical, and chemical treatments were implemented on individual 30 m x 11 m plots at each experimental site: 1) Prescribed burn with imazapic and glyphosate (Fall 2016; OSU-funded); 2) Imazapic and glysphosate (Fall 2016; OSU-funded); 3) Disking (Spring 2017; OSU-funded), 4) Prescribed burn (Fall 2017; Oregon Beef Council (OBC) funded); and 5) Modified rangeland drill (to be completed November 2017; OBC & OSU-funded). These treatments, and one other 30 m x 11 m control plot, are scheduled to be seeded at 12 lb/acre pure live seed with native (bluebunch wheatgrass and bottlebrush squirreltail; OBC-funded) and introduced (desert & Siberian wheatgrass; OBC-funded) grasses the week of November 6th—using OBC funds. During Winter 2017, Wyoming big sagebrush will be broadcasted on native experimental plots while forage kochia will be broadcasted on introduced seeding treatments (Figure 1). In spring/summer 2018, an undergraduate student will

1. This document is part of the Oregon State University – 2017 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.\

clip forages to assess crude protein and fiber of new growth as well as measure plant community cover and density.

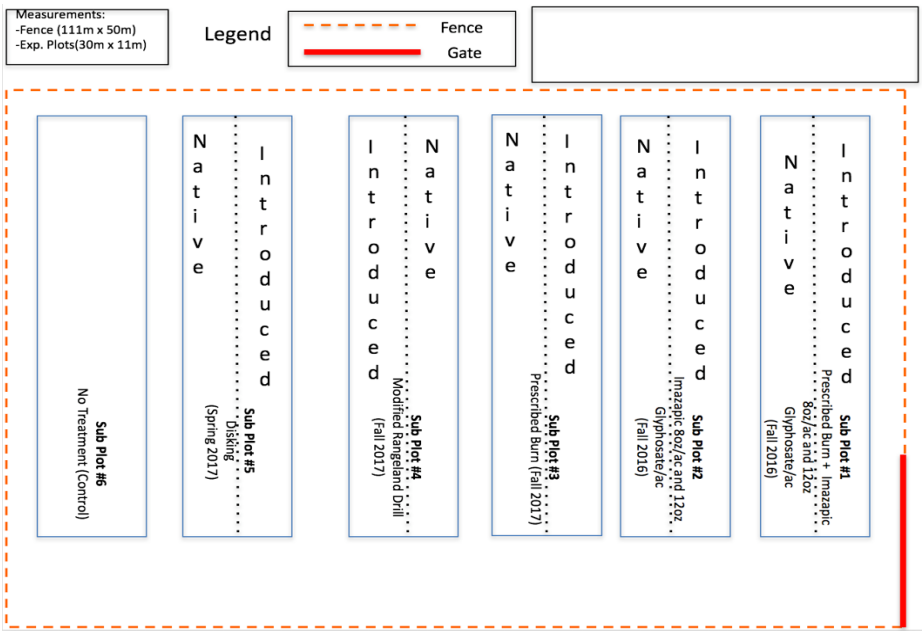

Figure 1: Developing conservation measures fire, mechanical, and chemical treatments and seeding treatments**.**

Evaluation of stubble height relationships to riparian health and function

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Project Objectives: To evaluate the relationship between stubble height and stream and riparian function and health metrics in a public cattle grazing management scenario in order to better inform cattle grazing systems in allotments that contain at-risk or federally-listed endangered species. Specifically, we seek to: (1) determine the relationship between stubble height and woody plant use by ungulates (cattle vs. elk/deer), woody plant recruitment and structural development in riparian areas, (2) determine the relationship between stubble height and streambank alteration due to ungulate trampling (cattle vs. elk/deer) over time, (3) evaluate the relationships between stubble height along the greenline and vegetation characteristics in the floodplain, and (4) document the relationship of stubble height with time spent by cattle and deer/elk in riparian areas.

Project Start Date: May of 2017
Expected Project Completion Date: October of 2020

Project Status: The first year of our study along 8 miles of Meadow Creek within the Starkey Experimental Forest and Range in the Blue Mountains in northeastern Oregon (Union County) was completed in October 2017. We collected data on greenline stubble height, utilization, vegetation composition, woody plant use, streambank alteration, and a number of salmonid habitat and stream characteristics within our five-pasture deferred rotation grazing system. Data from the first year of grazing is currently being analyzed. Fieldwork will continue for three more years. We expect this work inform management policy regarding cattle grazing on public allotments associated with salmonid-bearing streams. Grazing management is changing in the riparian areas of these allotments in response to regulations that are currently being reviewed and revised. The evolving direction for riparian grazing management would benefit from more definitive knowledge that this research should provide, and we expect that results will be useful in the development of improved riparian grazing policies/terms and conditions.

Organic Fertility Effect on Alfalfa Yield, Quality, Nutrient Concentration and Uptake, and Soil Fertility in Central Oregon

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Project Objectives: To test the effect of beef feedlot manure, and chicken manure, with and without some of the numerous different organic fertility enhancing products marketed today, on organic Alfalfa forage production. We will document effect on yield, quality, nutrient concentration, nutrient uptake, and soil fertility. (Some of these organic fertility enhancing products claim that after being applied the first year, and with continued annual or biannual application, other fertilizer nutrient needs will be cut in half the second year and beyond. Some of the products make the claim they will enhance the normal fertility program.)

Project Start Date: Fall of 2016
Project Completion Date: Fall of 2019

Project status: Preliminary alfalfa and weed yield has been compiled for both organic alfalfa fertility trials "I" (18 treatments) and trial "II" (12 treatment) comparisons that were conducted on the Dan Bansen Farm, Fort Rock, Oregon (one mile east of Fort Rock). Trial I (2012-2015) and Trial II (2013-2015) field work has been completed. Cost of these treatments ranged from $0/acre for the check to $450/acre for the sulfate of potash and bone meal treatment. Presently plant samples and soil samples have been ground and are being analyzed at the new and updated Plant and Soil Analytical Lab at the Central Analytical Lab at OSU. (Originally, we were going to run the soil samples at AgSource at Umatilla, OR and the plant samples at Brookside Lab, OH). The processing and testing really slowed down because it is a new lab, depends on students (no students during summer break), and the lab had trouble with one key analytical machine during start up. The 2015 soil and plant nutrient results should be ready in November / December, 2017.

The final trial year (2015) soil sample testing is being funded by Oregon Dairy Farmers Association and the final trial year (2015) plant sample nutrient testing is being funded by Oregon Beef Council. All replications will be tested for the last year for both of these trials to determine statistical difference between treatments or lack thereof. Previous years soil and plant sample replications will be pooled for testing to determine trends because of sheer cost. More grants will be sought based on the 2015 crop year test results, to continue working our way through testing the previous year's samples (2012-2014).

Treatments (18) in "Trial I" include:
+ Ocean Solutions with 2 and 4 ton/ac chicken manure
+ Excellerite with 2 and 4 ton/ac chicken manure
+ Symbex + Symbooster, with 2 and 4 ton chicken manure
+ Cascade Mineral with 2 and 4 ton beef cow manure
+ 2, 3, and 4 ton/acre chicken manure
+ 2, 3, and 4 ton/acre beef feedlot manure
+ Gypsum only
+ ½ X and 1X sulfate of potash + bone meal
+ Check

Treatments (12) in "Trial II" include:
+ Natura Sumagrow with 2 and 4 ton/ac chicken manure
+ Sea-90 with 2 and 4 ton/ac chicken manure
+ Humic Acid with 2 and 4 ton/ac rates of chicken manure
+ Accomplish with 2 ton of Chicken manure
+ Organic Digester with 0 and 2 ton chicken manure
+ 2 and 4 ton/acre chicken manure
+ Check

Preliminary Results

The preliminary four- and three-year biomass yields for the two trials are in the following four tables 1 - 4. At first glance there appears to be differences between treatments for first cutting total biomass yield; and there is, but, some of the treatments increased the broadleaf and grass weed yield substantially on first cutting each year. The increased weed pressure on first cutting did decrease yields in some treatments on second cutting yield, some

years. Third cutting yields for the treatments were all much the same. Second and third cuttings were relatively weed-free, except for the 2015 crop year, as grass weed yields increased. We documented weed pressure in some 7 and 3 treatments in trials "I" and "II", respectively since this was an organic alfalfa field. The amount of weed pressure on first cutting was amazing. Weed seed (mostly mustard and shepherdspurse) scattered in year one of the trial, blanketed the area.

The trials are a bit complicated. There appears to be no differences in alfalfa yield between the fertility enhancing products, but much work remains to determine other factors that will determine the most economic treatment, which appears to be the check at this point. Soil fertility at this site may have been adequate for the duration of the trials? Control of rodents (voles, pocket gophers, and Beldings' ground squirrels) was a huge challenge all 4 years!

Insect presence, not numbers, was documented in the first 3 years of the trials. Also because of rain delays in baling 2nd cutting, in 2012 and 2013 in trial "I", the effect of harrow bed wheel traffic was documented to decrease biomass yield, the width of the tire, slightly less than a ton per acre.

There appeared to be no quality differences between the original "priming" treatments in 2012 for trial "I".. Those original 9 treatments were split into 18 treatments in trial "I" during trial years 2013 -2015. The original 6 treatments used to "prime" trial "II" were split into 12 treatments for the 2014-2015 trial years.

Table 1. Preliminary 2012-2015 total dry matter yield for the organic alfalfa fertility trial "I" at the Dan Bansen farm at Fort Rock, Oregon.

Treatment and Rates	1st Cut Yield	2nd Cut Yield	3rd Cut Yield	Total Yield
	(tons/acre)			
Check (3 t/ac CM)	8.95	7.10	5.01	21.06
Beef Feedlot Manure (3 t/ac)	9.84	7.34	5.44	22.62
Ocean Solutions (1 gal/ac + Chicken Manure 2 t/ac)	9.97	6.65	5.29	21.91
Ocean Solutions (1 gal/ac + Chicken Manure (4 t/ac CM)	11.01	6.46	5.41	22.88
Chicken Manure (2 t/ac)	9.94	7.03	5.44	22.41
Chicken Manure (4 t/ac)	10.78	6.51	5.44	22.73
Beef Feedlot Manure (2 t/ac)	9.68	6.85	5.51	22.04
Beef Feedlot Manure (4 t/ac)	9.75	7.08	5.51	22.34
Symbooster/(13 oz/ac) + Symbex (2 qt./ac) + Chicken Manure 2 t/ac)	10.17	6.85	5.43	22.45
Symbooster/(13 oz/ac) + Symbex (2 qt./ac) + Chicken Manure 2 t/ac)	11.38	6.56	5.44	23.38
Excelerite (500 lb/ac) + Chicken Manure (2 t/ac)	9.87	6.72	5.40	21.99
Excelerite (500 lb/ac) + Chicken Manure (4 t/ac)	10.49	6.87	5.46	22.82
Gypsum (167 lb/ac)	8.92	6.82	5.21	20.95
Chicken Manure (3 t/ac)	10.09	6.84	5.26	22.19
Bonemeal (200 lb/ac) + Sulfate of Potash (200 lb/ac)	9.23	7.18	5.34	21.75
Bonemeal (400 lb/ac) + Sulfate of Potash (400 lb/ac)	9.04	7.10	5.51	21.65
Cascade Mineral (250 lb/ac) + Beef Feedlot Manure (2 t/ac)	8.83	7.14	5.40	21.37
Cascade Mineral (250 lb/ac) + Beef Feedlot Manure (4 t/ac)	9.07	7.13	5.50	21.70
Mean	9.83	6.90	5.39	22.12

All of the treatments had 3 ton/acre chicken manure applied at the beginning of the trial in 2012. These treatments were "primed" in 2012 and then fully implemented in 2013. Ocean Solutions was applied every two weeks from 2013-2015

Table 2. 2013 preliminary first cutting percent alfalfa, broadleaf weeds, and grass weeds in different treatments of the organic alfalfa fertility trial "I" at the Dan Bansen farm at Fort Rock, Oregon. (*An example of weed pressure on first cutting in one year.)*

Treatment and Rates	Mustard Stems	Broadleaf Weeds	Grass Weeds	Total Weeds	Alfalfa	Alfalfa Stems
	(#/ft2)	(%)				(#/ft2)
Check	23.1	42.6	0.0	42.6	57.4	58.3
Beef Feedlot Manure (2 t/ac)	19.4	51.7	0.0	51.7	48.3	44.5
Beef Feedlot Manure (3 t/ac)	22.5	64.6	0.0	64.6	35.4	40.6
Beef Feedlot Manure (4 t/ac)	15.1	63.6	0.0	63.6	36.4	40.5
Chicken Manure (2 t/ac)	19.1	59.7	0.0	59.7	40.3	46.9
Chicken Manure (3 t/ac)	20.1	66.3	0.0	66.3	33.7	39.5
Chicken Manure (4 t/ac)	19.2	68.3	0.0	68.3	31.7	39.2
Mean	*20.2*	*59.5*	*0.0*	*59.5*	*40.5*	*44.2*

Table 3. 2013-2015 preliminary total biomass yield for the organic alfalfa fertility trial "II" at the Dan Bansen farm at Fort Rock, Oregon.

Treatment and Rates	1st Cut Yield	2nd Cut Yield	3rd Cut Yield	Total Yield
	(tons/acre)			
Check	7.82	5.09	3.71	16.62
Accomplish (1 gal/ac) Beef Feedlot Manure (2 t/ac)	8.06	5.08	3.82	16.96
Chicken Manure (2 t/ac)	8.22	5.07	3.99	17.28
Chicken Manure (4 t/ac)	8.63	4.97	4.04	17.64
Natura Sumagrow (1gal/ac) Chicken Manure (2 t/ac)	8.13	5.31	4.09	17.53
Natura Sumagrow (1gal/ac) Chicken Manure (4 t/ac)	8.56	5.09	3.99	17.64
Humic Acid (10 lb/ac) Chicken Manure (2 t/ac)	8.80	4.97	4.06	17.83
Humic Acid (20 lb/ac) Chicken Manure 4 lb/ac)	8.79	5.11	4.05	17.95
Organic Digester (1 pt./ac) Chicken Manure (0 t/ac)	8.22	5.01	3.81	17.04
Organic Digester (1 pt./ac) Chicken Manure (2 t/ac)	8.71	4.93	4.00	17.64
Sea 90 (50+5+5+5 lb/ac) Chicken Manure (2 t/ac)	8.56	5.39	4.16	18.11
Sea 90 (50+5+5+5 lb/ac) Chicken Manure (4 t/ac)	9.08	5.23	4.01	18.32
Mean	8.47	5.10	3.98	17.55

Table 4. 2014 Preliminary first cutting percent alfalfa, broadleaf weeds, and grass weeds in different treatments of the organic alfalfa fertility trial "II" at the Dan Bansen farm at Fort Rock, Oregon. *(Example of second year weed pressure on first cutting in one year.)*

Treatment and Rates	Mustad Stems	Broadleaf Weeds	Grass Weeds	Total Weeds	Alfalfa	Alfalfa Stems
	#/ft2	(%)				(#/ft2)
Check	21.3	34.5	0.0	34.5	65.5	63.4
Chicken Manure (2 t/ac)	31.6	40.0	0.0	40.0	60.0	64.2
Chicken Manure (4 t/ac)	48.0	49.7	0.0	49.7	50.3	57.2
Mean	33.7	41.4	0.0	41.4	58.9	61.6

How much water do mature and juvenile juniper trees really use?

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Co-investigators: Mohamed A.B. Abdallah and Carlos Ochoa

Project Objectives: One factor related to juniper encroachment, water use by mature and juvenile juniper trees, has not been adequately studied. Because of this, it is not clear how much water are land managers saving by controlling juniper and how much of this savings are curtailed by the reestablishment of juvenile juniper trees that happens after years of juniper control. Therefore, our objectives were 1) To determine water use by mature juniper trees, 2) To determine water use by juvenile juniper trees that result after juniper control, and 3) To determine water savings in areas with juniper control after regrowth with respect to areas with intact mature juniper encroachment.

Project Start Date: We have installed automated water use equipment to monitor whole plant water use in mature trees in a watershed area where juniper has not been controlled. We have also installed automated water use equipment to evaluate whole plant transpiration in juvenile trees in a watershed area where juniper was controlled in 2005. In this area, the juvenile trees are resulting as regrowth after years of no control. The installation of the water use equipment was done in the fall of 2016 and we have been collecting data throughout 2017. The water use equipment that we have used is based on sap flow measurements by heat transfer.

Expected Project Completion Date: Water use data collection will continue through the summer of 2018. After that, the data will be summarized and reported.

Project Status: Water use data has been collected from May to October in mature juniper trees and from June to October in juvenile regrowth trees. In mature trees water use was low in May and June and it peaked during July and August to about 70-80 liters per day (Fig. 1). Subsequently, water use declined again in September and October. In small regrowth trees water use followed a similar seasonal pattern with high water use in July and August and low water use in September and October (Fig. 2). However, the amount of water used by small trees was always lower than 2 liters per day. In general, large trees used about 50 times more water than small trees. We would like to emphasize that these data are preliminary; we plan to continue water use data collection throughout 2018 and to determine the sapwood area of the measured trees at the end of 2018. That would help us to better determine water use and provide a more complete analysis of our data implications.

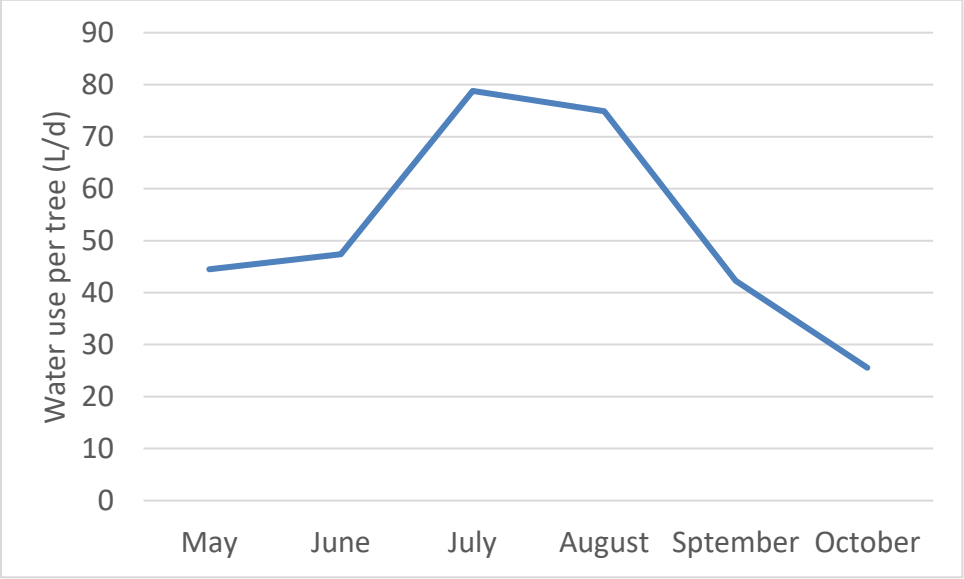

Figure 1. Water use by whole tree in large mature juniper trees in 2017.

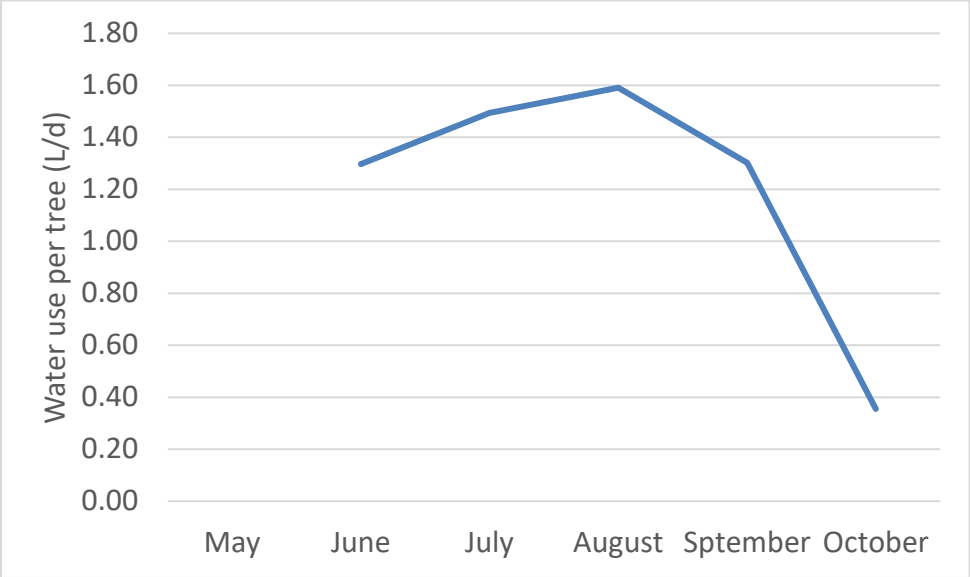

Figure 2. Water use by whole tree in small regrowth juniper trees in 2017.

Greater sage-grouse habitat suitability and management on historical crested wheatgrass seedings in southeastern Oregon

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Project Objectives: The practice of reseeding sagebrush dominated rangelands was the most common historically management practice and is still employed today. Unfortunately, 50 years after the mechanical and chemical manipulations of these sagebrush communities, very little is known about how these historical seedings serve as sage-grouse habitat.

This study will has two primary objectives: 1) to assess the current habitat suitability for sage-grouse on historical seedings of the Vale Rangeland Rehabilitation Project and other seedings over breeding, early brood-rearing, late brood rearing and fall/winter season uses and, 2) to examine the underlying conditions creating this variation in habitat suitability to assist in management.

Without better knowledge of how underlying conditions within seedings affect sage-grouse habitat today, we may misinterpret the drivers of change and diversity. For example, we may assume that grazing practices are responsible for current conditions, when in fact soil type and historical seeding implementation may be the drivers of the current vegetation composition.

Project Start Date: September of 2016
Expected Project Completion Date: October of 2018 (Request a one year extension)

Project Status: I hired an undergraduate student majoring in Rangeland Sciences to assist this fall with the collation of data and mapping using ArcGIS. Field data collection was postponed until spring and summer of 2018. I requested a one-year no cost extension to complete the grant due to health problems. Data analysis and a write-up of the final report should be completed by October 2017.

Preventing juniper reestablishment into sagebrush communities: Improving the watershed function.

Contact Person: Carlos Ochoa
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Project Objectives: 1) To characterize the progression of juniper re-occupation of sagebrush communities ten-years after tree removal, and; 2) To evaluate potential impacts of preventing juniper re-occupation on sagebrush steppe vegetation and hydrologic dynamics.

Project Start Date: 2017
Expected Project Completion Date: 2019

Project Status: We continue field data collection and analysis of historic vegetation and hydrologic data collected at the study site. Ongoing project results indicate tree density in treated and untreated watersheds are nearly the same (46 trees/acre). Juniper saplings in treated watershed range from 2 to 15 years old. Juniper canopy cover intercepts up to 46% of total annual precipitation. Overall, greater springflow, streamflow, and grass cover was observed in the treated watershed.

Seasonal aquifer recharge in the entire watershed-riparian system ranges from 2.5 to 4.5 ac-ft/ac, and it is heavily dependent on snowpack.

Research activities are following their expected course, this update relates to ongoing work in year 2, out of a 3-year study. This project will add critical information regarding how quickly juniper moves back into a treated site. Also, new data collected will allow us to enhance understanding of sagebrush steppe vegetation and water relationships in treated versus non-treated sites.

REPORT STATUS OF STUDIES FUNDED BY THE OREGON BEEF COUNCIL

Progress report not required for studies funded prior to 2010-2011 FY and with a full report submitted.

Projects funded in 2007 – 2008 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Wolf impact on cattle productivity and behavior	D. E. Johnson		**X**
Development of digital charting system for range health	D. E. Johnson		**X**
Livestock, plant community, and sage-grouse food sources	J. Miller		**X**
Animal Sciences			
Digestibility of cool-season in dairy farms	T. Downing		**X**
Female hormones and immune cells in cattle	M. Cannon		**X**
Diagnostic test for pregnancy detection in cattle	F. Menino		**X**
Assay to assess bovine embryo viability during transfer	F. Menino		**X**
Farm-based livestock manure/biogas production	M. Gamroth		**X**
Glycerol supplementation to cattle	C. Mueller		**X**
Copper and Zinc in dairy forage systems	T. Downing		**X**

Projects funded in 2008 – 2009 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Wolf impact on cattle productivity and behavior (cont.)	D. E. Johnson		**X**
Rangeland vegetation and sediment monitoring	L. Larson	**X**	**X**
Animal Sciences			
Late gestation protein supplementation of beef cows	D. Bohnert		**X**
Grazing options with *Brassicas* and Fodder Radishes	C. Engel		**X**
Maternal marbling potential and ultrasound technology	C. Mueller		**X**
Replacement heifers sired by high or low-marbling bulls	C. Mueller	**X**	**X**
BVDV and BVDV PI screening to initiate BVDB control	B. Riggs		**X**
Selenium supplementation and retention in beef cattle	G. Pirelli	**X**	**X**
Farm-based livestock manure/biogas production (cont.)	M. Gamroth		**X**

Projects funded in 2009 – 2010 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Wolf impact on cattle productivity and behavior (cont.)	D. E. Johnson		**X**
DNA analysis for cattle diet in sagebrush rangelands	R. Mata-Gonzales	**X**	**X**
Behavior and distribution of cattle grazing riparian zones	D.E. Johnson		**X**
Animal Sciences			
PFG2α to improve uterine health and reproductive efficiency	M. Cannon		**X**
Disposition and reproductive performance of brood cows	R. Cooke	**X**	**X**
Acclimation to handling and heifer development	R. Cooke	**X**	**X**
Farm-based livestock manure/biogas production (cont.)	M. Gamroth		**X**

Projects funded in 2010 – 2011 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Conflict stressors, spatial behavior and grazing budgets of cattle	D. E. Johnson	**X**	**X**
Behavior and distribution of cattle grazing riparian zones **(cont.)**	D. E. Johnson		**X**
Grazing and medusahead invasion in sagebrush steppe	D. D. Johnson	**X**	**X**
Weeds to suppress cheatgrass and medusahead	P. Dysart	**X**	**X**
Effects of wolves on cattle production systems **(cont.)**	D. E. Johnson		**X**
Quantities diet analysis in cattle using fecal DNA	R. Mata-Gonzales	**X**	**X**
Animal Sciences			
Protein supplementation to low-quality forage	D. Bohnert	**X**	**X**
Disposition, acclimation, and steer feedlot performance	R. Cooke	**X**	**X**
Nutrition during bull development on calf performance	C. Mueller	**X**	**X**
Extending grazing season with warm season and Brassica forages	S. Filley	**X**	**X**
Oral Selenium drench at birth to calves	J. Hall	**X**	**X**

Projects funded in 2011 – 2012 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Revegetating sagebrush rangelands Invaded by Medusahead	D. D. Johnson	**X**	**X**
Potential benefits of Sagebrush consumption by cattle	R. Mata-Gonzales	**X**	**X**
Effect of wolves on cattle production systems **(cont.)**	D. E. Johnson		**X**
Conflict stressors, spatial behavior and grazing budgets **(cont.)**	D. E. Johnson	**X**	**X**
Animal Sciences			
Effects of camelina meal supplementation to beef cattle	R. Cooke	**X**	**X**
The economics of grassed-based dairying in Oregon	T. Downing	**X**	**X**
Yeast culture supp. improves feed consumption in cattle	G. Bobe	**X**	**X**
Western Juniper - Induced Abortions in Beef Cattle	C. Parsons	**X**	**X**

Projects funded in 2012 – 2013 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Effect of wolves on cattle production systems (cont.)	D.E. Johnson		**X**
Modification of livestock and sage-grouse habitat after juniper control	R. Mata-Gonzales	**X**	**X**
Prescribed burning and herbicide appl. to revegetate rangelands	D. D. Johnson	**X**	**X**
Animal Sciences			
Comparison of Ivomec Plus and a generic anthelmintic to beef cattle	R. F. Cooke	**X**	**X**
Influence of supplement composition on low-quality forages	D. W. Bohnert	**X**	**X**
Yeast culture supplementation and dairy reproductive performance	G. Bobe	**X**	**X**
The effect of western juniper on the estrous cycle of beef cattle	C. Parsons	**X**	**X**

Projects funded in 2013 – 2014 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Development of forage value index for Ryegrass	T. Downing	**X**	**X**
Effect of wolves on cattle production systems (cont.)	J. Williams		**X**
Use of herbicide for control of Western Juniper	G. Sbatella		**X**
Animal Sciences			
Oxidized lipid metabolites to predict disease in dairy cows	G. Bobe	**X**	**X**
Cow nutritional status during gestation and offspring performance	R. F. Cooke	**X**	**X**
Modifying the hormone strategy for superovulating donor cows	F. Menino	**X**	**X**

Projects funded in 2014 – 2015 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Development of forage value index for Ryegrass	T. Downing	**X**	**X**
Research on stream water temperature and sediment loads	C. Ochoa	**X**	**X**
Techniques to improve seedling success of forage kochia	D. D. Johnson	**X**	
Animal Sciences			
Identification of predictive metabolomics markers in dairy cows	G. Bobe	**X**	**X**
Cow nutritional status during gestation and offspring performance	R. F. Cooke	**X**	**X**
Modifying the hormone strategy for superovulating donor cows	F. Menino	**X**	**X**
Energetic output of beef cows based on lactation and calf crop	C. Mueller	**X**	
Influence of supplement type and monensin on forage utilization	D. W. Bohnert	**X**	**X**

Projects funded in 2015 – 2016 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Research on stream water temperature and sediment loads	C. Ochoa	**X**	**X**
Impacts of wolf predation on stress in beef cattle	R. Cooke	**X**	**X**
Techniques to improve seedling success of forage kochia	D. D. Johnson	**X**	**X**
Animal Sciences			
Modulation of milk fat synthesis in dairy animals	M. Bionaz	**X**	**X**
Peripartal vitamin E injections prevent diseases in dairy cows	G. Bobe	**X**	
Cow nutritional status during gestation and offspring performance	R. Cooke	**X**	**X**
Development of enhanced cattle embryo transfer medium	A. Menino	**X**	**X**
Energetic output of beef cows based on lactation and calf crop	C. Mueller		

Projects funded in 2016 – 2017 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Preventing juniper reestablishment into sagebrush communities	C. Ochoa	**X**	**X**
Research on stream water temperature and sediment loads	C. Ochoa	**X**	**X**
Greater sage grouse response to landscape level juniper removal	C. Hagen	**X**	**X**
Greater sage grouse habitat suitability and management in SE Oregon	L. Morris	**X**	
Organic fertility effect on alfalfa hay in Central Oregon	M. Bohle	**X**	
Animal warm season grasses for forages	G. Wang	**X**	**X**
Animal Sciences			
Peripartal vitamin E injections prevent diseases in dairy cows	G. Bobe	**X**	
Feeding immunostimulants to enhance receiving cattle performance	R. Cooke	**X**	**X**
Development of enhanced cattle embryo transfer medium	A. Menino	**X**	**X**
In vivo-in vitro hybrid system to perform nutrigenomic studies in cattle	M. Bionaz	**X**	
Feeding Se-fertilized hay to reduce parasite load in beef calves	J. Hall	**X**	
Evaluation of biological deterrents to manage wolf movements	M. Udel	**X**	**X**

Projects funded in 2017 – 2018 FY

Abbreviated Project Title	Senior Investigator	Report Status Progress	Full
Rangeland Ecology and Management			
Preventing juniper reestablishment into sagebrush communities	C. Ochoa	**X**	
Conservation measures to restore rangeland on sage-grouse habitat	S. Arispe	**X**	
How much water do mature and juvenile juniper trees need?	R. Mata-Gonzales	**X**	
Evaluation of stubble height relationship to riparian health and function	B. Endress	**X**	
Animal Sciences			
Development of enhanced cattle embryo transfer medium	A. Menino	**X**	
Feeding essential fatty acids to late-gestating cows	R. Cooke	**X**	
Impacts of estrus expression and intensity on fertility of beef cows	R. Cooke	**X**	
Increasing milk production in bovine mammary cells	M. Bionaz		**X**
Identification of cyanobacterium in Lake county	T. Dreher	**X**	
Use of platelet rich plasma for endometritis in beef heifers	M. Kutzler	**X**	

Oregon State University

Beef Cattle Sciences