

Beef Cattle Sciences

Oregon Beef Council Report

2018 Edition

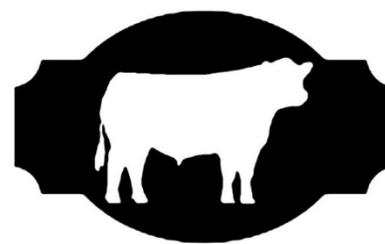


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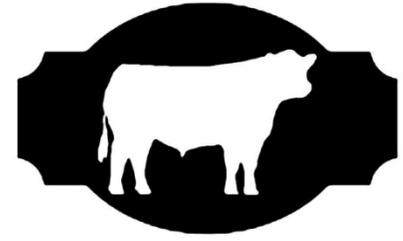


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Oregon Beef Council

Report

Beef Cattle Sciences

Effects of prostaglandin F_{2α} administration 24 h prior to CIDR removal to increase estrus expression, intensity, and fertility in beef cows¹

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Synopsis

Administration of prostaglandin F_{2α} 24 h prior to CIDR removal increases expression of estrus in suckled beef cows assigned to a 7-d CO-Synch + CIDR program.

Summary

The objective was to evaluate if administration of PGF_{2α} 24 h prior to CIDR removal induces a more rapid decrease in circulating progesterone (P₄) and consequently increases the proportion of cows exhibiting high-intensity estrus prior to fixed-time artificial insemination (FTAI). Angus-influenced cows received 100 µg of GnRH and a CIDR device containing 1.38 g of P₄ on d 0, and were randomly assigned to 25-mg injection of PGF_{2α} on d 6 (PG6; n=147) or d 7 (PG7; n = 162). The CIDR was removed on d 7, and cows received 100 µg of GnRH and FTAI on d 10. An estrus detection patch was attached to the tailhead of each cow on d 7, and estrus expression assessed at FTAI. Blood samples were collected on d 0 to determine the presence of a functional corpus luteum via P₄ (CL, P₄ ≥ 1.0 ng/mL; NOCL, P₄ < 1.0 ng/mL). Blood samples for P₄ analysis were collected on d 6, and on d 7 at CIDR removal and 1 h later. Plasma P₄ at CIDR removal and 1 h later were less in PG6 vs. PG7 cows classified as CL, and did not differ within NOCL ($P \geq 0.43$). Estrus expression was greater in PG6 vs. PG7 cows classified as CL (72.8 vs. 48.3%), but similar ($P = 0.$

96) within NOCL. Pregnancy rates did not differ between treatments ($P = 0.53$), cows classified as CL, and did not differ within NOCL ($P \geq 0.43$). Estrus expression was greater in PG6 vs. PG7 cows classified as CL (72.8 vs. 48.3%), but similar ($P = 0.96$) within NOCL. Pregnancy rates did not differ between treatments ($P = 0.53$), despite a numerical difference ($P = 0.15$) between PG6 vs. PG7 cows classified as CL (65.5 vs. 55.0%). Hence, PGF_{2α} administration 24 h prior to CIDR removal appears to benefit estrus expression and reproductive performance of beef cows with a functional CL at the beginning of the 7-d CO-Synch + CIDR protocol.

Introduction

The development of estrus synchronization protocol has enabled widespread adoption of artificial insemination (AI) and consequently has had major economic impact on the beef industry (Lamb and Mercadante, 2016). However, this technology is still underutilized by beef producers in the United States. According to NAHMS, 2008, only 7.6% of the beef operations in the country utilize AI, whereas 72.5% of the pregnancies in the dairy industry are generated through this biotechnology (NAHMS, 2009). Strategies that increase pregnancy rates and facilitate the adoption of AI can have major impacts on beef production efficiency.

The main aspect about estrus synchronization that enables the utilization of AI in large scale is the fact that ovulation can be induced in cows at a predetermined time regardless of whether they exhibit estrus behavior (Ryan et al., 1998). Hence, estrus

detection can be eliminated through the use of estrus synchronization and fixed-time AI (FTAI). However, cows that are exposed to FTAI without expressing estrus have decreased fertility (Perry et al., 2005; Thomas et al., 2014a). The expression of estrus behavior requires a decrease in circulating plasma concentrations of progesterone (P4), which allows for an increase in circulating estradiol (Vailes et al., 1992; Allrich, 1994). Therefore, estrus strategies that can induce a rapid decrease in circulating P4 prior to FTAI might result in more females exhibiting estrus and consequently higher pregnancy rates.

We hypothesized that the injection of prostaglandin F_{2α} 24 h prior to CIDR removal in a 7-d CO-Synch + CIDR can induce a more rapid decrease circulating P4, consequently altering the intensity of estrus expression in beef cows. This leads to an increase in the number of cows exhibiting estrus between CIDR removal and FTAI, and potentially benefits fertility. Therefore, the objective of this study was to evaluate the impacts of prostaglandin F_{2α} 24 h prior to CIDR removal on estrus expression and parameters associated with fertility in beef cows.

Materials and Methods

Three hundred and nine Angus-influenced suckled beef cows (n = 309; Table 1) from 3 different locations were submitted to the 7 d CO-Synch + CIDR estrus synchronization protocol followed by FTAI (Larson et al., 2006). Cows received 100 µg of GnRH and a CIDR device on d 0, and were randomly assigned to receive a 25 mg injection of PGF_{2α} on d 6 (PG6; n=147) or d 7 (CON; n = 162). The CIDR device was removed on d 7 and an estrus detection patch was attached to the tail head of each cow. Estrus patch activation was assessed at FTAI. Patches were considered activated when at least 50% of the patch was discolored, or when the patch was absent. Cows received 100 µg of GnRH concurrent with FTAI, which was performed 63 ± 3 h after CIDR removal. Pregnancy diagnosis was performed 30 d after FTAI by transrectal ultrasonography luteum using an Ibex ultrasound equipped with a linear 5 MHz multifrequency transducer.

Blood samples were collected on d 0 from all cows to determine the presence of a functional corpus luteum (CL) via plasma concentration of P4. Cows were considered to have a functional CL if plasma concentrations of P4 were ≥ 1.0 ng/mL. Cows with plasma concentrations of P4 < 1.0 ng/mL were classified as not having a functional CL. Blood samples for plasma P4 analysis were also collected in

one of the locations (42 cows/treatment) on d 6, and on d 7 at CIDR removal and 1 h later. The rationale for measuring P4 1 h after CIDR removal was to better assess the endogenous circulating concentrations of P4, since the P4 coming from the CIDR insert should be, to some extent, already metabolized at that point. All samples were collected via venipuncture from the coccygeal vein. Samples were immediately placed on ice and then centrifuged at 2000 g for 15 min for plasma separation. Aliquots were transferred to polypropylene vials and stored at -20°C. Plasma samples were analyzed for P4 concentrations using a chemiluminescent enzyme immunoassay (Immulite 2000 XPi platform; Siemens Medical Solutions USA, Inc.).

Quantitative (BW, BCS, days postpartum, plasma concentration of P4) and binary (presence of CL on d 0, estrus expression, and pregnancy outcomes) data were analyzed with the MIXED and GLIMMIX procedures of SAS (SAS Inst., Inc., Cary, NC, USA), respectively. The model utilized for analyzing BW, BCS, days postpartum, and CL on d 0 included the fixed effect of treatment and the random effects of location and cow(treatment × location). The analysis of estrus expression and pregnancy rates included the fixed effects of treatment, CL on d 0, and their respective interaction. Location and cow(treatment × location × CL on d 0) were included as random effects. When analyzing plasma concentration of P4, treatment, CL on d 0 and their respective interactions, were included as fixed effects. The random effect of cow (treatment × CL on d 0) we also included in the analysis. Significance was set at P ≤ 0.05.

Results

There were no differences between treatments on cow BW, BCS, days postpartum, and on the percentage of cows with a functional CL on d 0 (P ≥ 0.38; Table 1). Plasma concentrations of P4, estrus responses and pregnancy rates are summarized in Table 2. As expected, cows with a functional CL on d 0 had greater (P < 0.01) plasma concentrations of P4 at d 6 when compared to cows without a functional CL (4.82 vs. 2.64 ± 0.488 ng/mL, respectively). In addition, there were no differences between treatments (P = 0.80) or a treatment × CL on d 0 interaction (P = 0.23) on plasma concentrations of P4 at d 6. However, a treatment × CL on d 0 interaction (P < 0.03) was observed at the time of CIDR removal, where PG7 cows that had a functional CL at d 0 had greater plasma concentrations of P4 compared to PG6 cows that had a CL at d0 (P < 0.01). As expected, plasma concentrations of P4 were similar between

PG6 and PG7 cows that did not have a CL on d 0 ($P = 0.43$). In blood samples collected 1 h after CIDR removal, similar results were observed. There was also a treatment \times CL on d0 interaction ($P < 0.02$), where cows that had a functional CL on d 0 and received PGF2 α 24 h earlier had lower concentrations of P4 compared to PG7 cows that had a CL ($P < 0.01$). Treatments did not alter concentrations of P4 1 h after CIDR removal in cows that did not have a CL on d 0 ($P = 0.54$).

The overall percentage of cows exhibiting estrus between CIDR removal and FTAI was 64.1% (198/309). Cows that exhibited estrus had 17.3 % greater pregnancy rates to FTAI than cows that were not in estrus. When evaluating the effects of treatment on estrus expression, a treatment \times CL on d 0 interaction was observed ($P < 0.03$). The administration of PGF2 α on d 6 resulted in a greater ($P < 0.01$) estrus response within cows that had a functional CL on d 0 compared to cows that received PGF2 α at the time of CIDR removal. Estrus expression in cows that did not have a functional CL on d 0 were not altered by treatment ($P = 0.87$). Overall pregnancy rates to FTAI were 57.5 % (177/308). As expected, although treatments had major impacts on circulating concentrations of P4 and estrus expression prior to FTAI, there were no effects of treatment ($P = 0.54$), presence of CL on d 0 ($P = 0.28$), nor treatment \times CL on d 0 interaction ($P = 0.23$) on the percentage of cows pregnant by FTAI. However, it is important to mention that this experiment was not designed to detect a statistical differences in pregnancy rate. In fact, when analyzing pregnancy rates within the cows that had a CL on d 0, PG6 cows had a numerical increase of 10.5 % in pregnancy rates compared to PG7 cows, indicating that PG6 treatment might have the potential to increase pregnancy rates in a 7-d CO-Synch + CIDR program.

Conclusions

In conclusion, results from this experiment indicate that administration of prostaglandin F2 α 24 h prior to CIDR removal induces a more rapid decrease in circulating concentrations of P4, consequently increasing the expression of estrus between CIDR removal and FTAI in suckled beef cows that have a functional CL at the beginning of 7-d CO-Synch + CIDR program. This might lead to an increase in pregnancy rates to FTAI; however, further research is required with a greater number of animals in order to detect a statistical difference in fertility.

Acknowledgements

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Table 1. Body weight (**BW**), body condition score (**BCS**), days postpartum, and presence of a functional CL at the beginning of the estrus synchronization protocol.^{a,b}

Item	PG6	PG7	SEM	P =
BW, kg	557	560	10	0.69
BCS ^c	5.2	5.2	0.06	0.62
Days postpartum	74.3	73.0	2.6	0.55
CL on d 0, %	57.5 (84/147)	62.5 (100/162)	5.74	0.38

^a Cows were exposed to the 7 d CO-Synch + CIDR estrus synchronization protocol; however, prostaglandin F_{2α} injection was given 24 h prior to the removal of CIDR insert.

^b Cows were exposed to the a control treatment consisting on the conventional 7 d CO-Synch + CIDR estrus synchronization protocol (Larson et al., 2006).

^c Body condition score (1 to 9 scale; according to Wagner et al., 1988).

Table 2. Progesterone concentrations and reproductive outcomes according to treatment and CL on d 0.^{a,b}

Item	No CL on d 0		CL on d 0		SEM	Trt	Trt x CL
	PG6	PG7	PG6	PG7			
Plasma progesterone, ng/mL							
d 6	3.07	2.22	4.53	5.09	0.57	0.80	0.23
d 7 ^c	1.39	1.91	1.80 ^b	3.99 ^a	0.38	< 0.01	0.03
d 7 +1 h ^d	0.73	1.07	1.06 ^b	2.89 ^a	0.32	< 0.01	0.02
Reproductive responses							
Estrus expression, %	56.0 (28/63)	55.5 (28/62)	72.8 ^a (54/84)	48.3 ^b (44/100)	7.3	0.04	0.05
Pregnancy rates, %	52.4 (33/63)	55.7 (34/62)	65.5 (55/84)	55.0 (55/100)	5.7	0.54	0.23

^a Cows were randomly assign to the receive a prostaglandin F_{2α} injection on d 6 (PG6) or d 7 (PG7) of the 7 d CO-Synch + CIDR estrus synchronization protocol (Larson et al., 2006).

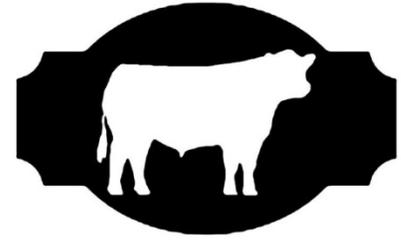
^b Within CL category, values with different superscripts (a,b) differ ($P < 0.05$).

^c Blood samples were collected at CIDR removal to determine circulating concentration of progesterone.

^d Blood samples were collected 1 h after CIDR removal to determine circulating concentration of progesterone.



Oregon Beef Council Report



Beef Cattle Sciences

Feeding Selenium-Biofortified Alfalfa Hay During The Preconditioning Period Improves Growth And Carcass Weight Of Beef Calves¹

Jean A. Hall²

Synopsis

Feeding Se-fertilized alfalfa hay for 9 weeks was effective at increasing whole-blood Se concentrations and body weights in the preconditioning period in weaned beef calves, and tended to be effective at increasing hot carcass weights in the feedlot.

Summary

The objective of this experiment was to show that feeding Se-biofortified alfalfa hay increases whole-blood (WB) Se concentrations, decreases gastrointestinal parasite load, and improves calf performance in the feedlot. Recently weaned beef calves (n=30) were randomly assigned to two groups with 3 pens of 5 calves per treatment group, and fed an alfalfa hay based diet for 9 weeks. Alfalfa hay was harvested from fields fertilized with sodium selenite at a rate of 0 or 89.9 g Se/ha. Calculated Se intake from dietary sources was 1.09 and 27.45 mg Se/calf/day for calves consuming alfalfa hay with Se concentrations of 0.06 and 3.47 mg Se/kg dry matter, respectively. Blood samples were collected at baseline and 3, 6, and 9 weeks and analyzed for WB-Se concentrations. The WB-Se concentrations after 9 weeks of feeding Se-enriched alfalfa hay were significantly higher in the treatment group (556±11 vs 140±11 ng/dL; P<0.001). Fecal samples were collected at baseline and after 5 and 9 weeks and counted for total trichostrongyle-type eggs and *Haemonchus contortus* eggs (in samples with ≥ 25

trichostrongyle-type eggs/g feces). Because fecal trichostrongyle-type egg counts were low, group differences were not apparent. Feeding Se-fertilized alfalfa hay was effective at increasing body weight (BW) in weaned beef calves ($P_{Treatment} = 0.03$) and tended to be effective at increasing hot carcass weight at wk 34 in the feedlot ($P_{Treatment} = 0.07$). Our results suggest that, in areas with low-forage Se concentrations, feeding Se-enriched alfalfa hay during the weaning transition period improves growth and final carcass weights of beef calves.

Introduction

Selenium (Se) is an essential trace mineral important for immune function and overall health of cattle. Optimal immune function is critical for calves undergoing the stresses of weaning, relocation to feedlots, and commingling with animals of different origins. The National Agriculture Statistics Service shows that 630,000 calves were born in Oregon in 2017. The majority of Oregon grown calves enter the feedlot. Even with good vaccination programs, producers often encounter significant health issues in the feedlot, including mortality. Reducing these losses by enhancing immune function would have a significant economic impact for Oregon cattle producers.

Selenium has been recognized for years as an essential trace element for animals. The Northwest region is among those with the lowest amounts of Se in soils and plants (Oldfield, 2001). In general, the majority of livestock raised in low-Se regions do not

receive sufficient dietary Se for optimum health. In Oregon, Se concentration of forages is lower than that required by livestock (Filley et al., 2007). Providing adequate Se is important to prevent Se-responsive diseases. Although the essentiality of Se supplementation has been known for five decades, the most effective method of Se delivery to livestock to achieve optimum performance is still being investigated. Attempts to provide supplemental Se to animals through trace minerals or injection usually fail to maintain blood Se concentrations necessary for optimal health and productivity. A promising Se supplementation method is Se fertilization, as it increases Se concentrations in plants, and in animals consuming the Se-biofortified forages and hay (Hall et al., 2011; Hall et al., 2013a).

Plants require sulphur, not Se, for amino acid production. In plants, however, Se is incorporated into methionine as selenomethionine, and when forage is consumed by livestock, Se from selenomethionine is incorporated into selenoproteins, whose functions range from antioxidant, anti-inflammatory, and detoxification to thyroid hormone activation. Nitrogenous fertilizers, widely hailed as one of the most important advances in agricultural technology, increase biomass but dilute essential minerals like Se, emphasizing the need for Se amendments (He et al., 2005). Application of Se directly to pastures and hayfields increases forage Se concentration in a dose dependent manner (Filley et al., 2007) and improves blood Se concentrations (Hall et al., 2009), animal performance (Hall et al., 2013a) and immunity (Hall et al., 2013b; Hall et al., 2013c).

As beef producers enter into niche markets with natural and grass fed beef, anthelmintic resistance and strict limits on the use of antibiotics emphasize the need for alternative methods to enhance cattle immunity, prevent disease, and improve production. The objectives of this study were to show that feeding Se-biofortified hay increases WB-Se concentrations, decreases gastrointestinal parasite load, and improves calf performance in the feedlot.

Materials and Methods

Animal ethics statement and study design

The experimental protocol was reviewed and this study was approved by the Oregon State University Animal Care and Use Committee (ACUP Number: 4883). This was a prospective clinical trial of 9-week duration (October 11, 2017 through December 11, 2017) involving 30 weaned beef calves, primarily of

Angus breeding. The study design consisted of 2 treatment groups, with three pens of five animals per treatment. The study was conducted at the Hogg Animal Metabolism barn on the Oregon State University campus (Corvallis, OR, USA).

The weaned beef calves at baseline ranged in age from 6 to 9 months and originated from the Oregon State University beef ranch, Corvallis, OR, USA. Body weights at baseline ranged from 240 to 334 kg (286 ± 9.3 kg, mean \pm SEM), and body condition scores ranged from 6 to 7 (1 to 9 scale). There were 30 steer calves in the study. Routine farm management practices, including vaccinations and deworming, were the same for both treatment groups.

Using a randomized complete block design, calves were blocked at the time of weaning by BW and then assigned to one of 2 treatment groups of 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). Pens provided 10 m²/calf of concrete flooring in open lots that were strip cleaned once weekly, 5 m²/calf of shavings in a loafing area, and 98 cm of feeder space/calf as concrete bunks. All measurements exceeded requirements (MWPS-6, 1987) with continuous access to water, feed bunks, and shelter.

Calves were fed a mixture of alfalfa and grass hay twice daily. The amount of hay fed was adjusted weekly to ensure that calves had all the hay they wanted for consumption yet with minimal wastage. The ration was formulated for growing beef calves in the 250 to 350 kg BW range to achieve a target average daily gain of 0.5 kg/day. The goal was to feed hay at a rate of 85% alfalfa and 15% grass hay. Calves were transitioned to their respective hay rations over a 3-week 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; 2.05 kg/head/day 4; 2.5 kg/head/day 5; 2.95 kg/head/day 6; and 3.41 kg/head/day 7. During the first week, grass hay was added to achieve a total hay intake of 6.82 kg/head/day. Thereafter, in week 2 the amount of alfalfa hay fed was increased from 3.41 to 5.91 kg/head/day. In week 3 alfalfa hay fed was increased to 6.36 kg/head/day and total hay intake was 7.73 kg/head/day. In weeks 4, 5, and 6 alfalfa hay fed was increased from 6.36 to 7.27 kg/head/day and total hay intake was increased from 7.73 to 8.64 kg/head/day. In weeks 7, 8, and 9 alfalfa hay intake was 7.73 kg/head/day and total hay intake was between 8.18 and 9.09 kg/head/day.

Also in week 3, 0.45 kg/head/day of a medicated grain-based concentrate was fed (OSU Steer-A-Year

Pellet R35; manufactured by CHS Inc., Sioux Falls, SD). This amount was increased to 0.68 kg/head/day in weeks 4 through 9. This feed contained a coccidiostat (monensin sodium, 35 g/ton). The grain pellets were fed once a day and consumed before hay was fed. The guaranteed analysis of grain pellets was 11.0% crude protein, 3.5% crude fat, and 6.0% crude fiber. The concentration of Se in the grain pellets was 0.77 mg/kg.

Prior to this study, calves had free-choice access to a mineral supplement containing 120 mg/kg Se from sodium-selenite. The mineral supplement (dry matter basis) was in loose granular format and contained 57.0 to 64.0 g/kg calcium; 30.0 g/kg phosphorus; 503 to 553 g/kg salt (NaCl); 50.0 g/kg magnesium; 50 mg/kg cobalt; 2,500 mg/kg copper; 200 mg/kg manganese; 200 mg/kg iodine; 6,500 mg/kg zinc (Wilbur-Ellis Company, Clackamas, OR). During the feeding trial, mineral supplement without Se was provided for free-choice consumption.

After the 9-week preconditioning period, calves were shipped to a commercial feedlot in Burbank, WA (Simplot Feeders, Pasco WA). Calves were examined and blood and fecal samples were collected 23 days after transfer to the feedlot. At the end of the 25-week feedlot period, calves were sent to a commercial meat packing plant (Tyson Fresh Meats Inc; Pasco, WA). Hot carcass weights, carcass quality grade (no-roll, standard, select, choice, prime), and yield grade (1 to 4) were recorded.

Selenium fortified-alfalfa hay

Third cutting alfalfa hay was enriched with Se by mixing inorganic sodium-selenate (RETORTE Ulrich Scharrer GmbH, Röthenbach, Germany) with water and spraying it onto the foliage (approximately 10 cm height) of an alfalfa field at application rates of 0 or 89.9 g Se/ha in July 2017. The application rate was chosen based on a previous study (Hall et al., 2013a). Third-cutting alfalfa hay was harvested early October 2017 and then analyzed for Se and nutrient content. A Penn State forage sampler was used to take 25 cores from random bales in each hay source (0 or 89.9 g Se/ha) prior to beginning the feeding trial. Core samples were mixed well and representative samples selected for Se analysis (Table 1; Utah Veterinary Diagnostic Laboratory, Logan, UT). Plant samples were prepared for Se analysis as previously described (Davis et al., 2012), and Se was determined using inductively coupled argon plasma emission spectroscopy (ICP-MS; ELAN 6000, Perkin Elmer,

Shelton, CT). Quantification of Se was performed by the standard addition method, using a 4-point standard curve. A quality-control sample (in similar matrix) was analyzed after every 5 samples, and Se analysis was considered acceptable if the Se concentration of the quality-control sample fell within $\pm 5\%$ of the standard/reference value for the quality control. Alfalfa hay samples were also submitted to a commercial laboratory for routine nutrient analysis (Table 1; Cumberland Analytical Services, Maugansville, MD). Alfalfa hay dry matter determination was completed at a temperature of 105 °C for 12 to 14 h in a forced draught oven. Methods for crude protein (CP), acid detergent fiber (ADF), ash, and minerals were performed according to the Association of Official Analytical Chemists (AOAC, 2000). The neutral detergent fiber (NDF) was determined according to Van Soest et al. (1991). Soluble protein was determined according to Krishnamoorthy et al. (1982).

Blood collection for selenium analyses

Blood samples were collected from the jugular vein of weaned beef calves, at baseline, after 3, 6, and 9 weeks of alfalfa hay consumption, and 3 weeks after transfer to the feedlot, into evacuated EDTA tubes (2 mL; final EDTA concentration 2 g/L; Becton Dickinson, Franklin Lakes, NJ) and stored on ice until they were frozen at -20 °C to measure WB-Se concentrations. Selenium concentrations were determined by a commercial laboratory (Utah Veterinary Diagnostic Laboratory, Logan, UT) using an inductively coupled argon plasma emission spectrometry method. Selenium was measured using an ICP-MS (ELAN 6000, Perkin Elmer, Shelton, CT) method as previously described (Hall et al., 2013a).

Fecal sample collection and analysis

Fecal samples were collected 30 days before the feeding trial began, after 5 and 9 weeks of alfalfa hay consumption, and again 3 weeks after transfer of calves to the feedlot. Fecal samples were extracted manually per rectum using a powder-free latex examination glove (Diamond Grip™ Latex Gloves, Microflex, Reno, NV) while calves were restrained in a chute. Fecal samples were stored in the glove at 4 °C until analysis. The minimum sample amount was 10 grams.

For quantification of fecal trichostrongyle-type eggs, we followed the McMaster egg count method of

Whitlock (1948). When at least 25 trichostrongyle-type eggs/g feces were observed, fecal samples were submitted to a commercial laboratory (Veterinary Diagnostics Laboratory, Oregon State University) and assayed for the presence of *H. contortus* eggs using a fluorescein-labeled peanut agglutinin test, following the procedure of Jurasek et al. (2010). The percentage of total ova that were positively identified as *H. contortus* was reported.

Statistical analysis

Statistical analyses were performed using SAS version 9.2 (SAS Institute, 2009). Data were analyzed as repeated-measures-in-time using PROC MIXED. Fixed effects in the model were Se application rate (0 and 89.9 g Se/ha), baseline values (as linear covariate), time (after 3, 6, and 9 weeks of feeding Se enriched hay), and the interaction between Se application rate and time. An unstructured variance-covariance matrix was used to account for variation of measures within calves. In addition, continuous data collected after the Se hay feeding period were analyzed in PROC GLM. Mean values were used for each pen, as pen was the experimental unit. Data are reported as least square mean \pm SEM. Statistical significance was declared at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

Fecal parasite egg counts were logarithmically transformed by base 10 to achieve normal distribution. Data were measured over time; thus, repeated-measures-in-time analysis was performed using PROC GLIMMIX assuming a negative binomial distribution. Fixed effects in the model were Se application rate (0 and 89.9 g Se/ha), baseline values (as linear covariate), time (after 5 and 9 weeks of feeding Se enriched hay), and the interaction between Se application rate and time. All statistical tests were two-sided. Data are reported as least square mean \pm SEM.

Results

Agronomic biofortification

Fertilizing the alfalfa hay field with sodium-selenate at 89.9 g/ha increased the Se content of third-cutting alfalfa from 0.06 (non-fertilized control) to 3.47 mg Se/kg dry matter. Calculated Se intake from alfalfa hay was 0.46 and 26.82 mg Se/calf/day, respectively, for calves consuming hay with Se concentrations of 0.06 and 3.47 mg Se/kg dry matter. The concentration of Se in the grass hay was 0.12

mg/kg. Calculated Se intake from grass hay was approximately 0.11 mg Se/calf/day. The measured Se concentration of the grain concentrate was 0.77 mg/kg dry matter. Calculated Se intake from grain concentrate was 0.52 mg Se/calf/day in weeks 7 through 9 of the preconditioning feeding period. Thus, total dietary Se intake during weeks 7 to 9 was 1.09 and 27.45 mg Se/calf/day, respectively for calves in control and high-Se treatment groups.

Whole-blood selenium concentrations

Feeding Se-fertilized alfalfa hay was effective at increasing WB-Se concentrations in weaned beef calves ($P_{Treatment}$, P_{Week} , and $P_{Interaction}$ all ≤ 0.004 ; **Figure 1**). The normal reference interval for WB-Se concentrations of adult cows is 120 to 300 ng/mL (Hall et al., 2011). The WB-Se concentrations continued to increase throughout the 9-week preconditioning period (wk 3: +50%; wk 6: +192%; wk 9: +292%). During the initial feedlot period (wk 12: +272%), WB-Se concentrations remained higher ($P_{Treatment} < 0.001$).

Body weights and carcass data

Feeding Se-fertilized alfalfa hay was effective at increasing BW in weaned beef calves ($P_{Treatment}$, = 0.03; P_{Week} , < 0.001 and $P_{Interaction}$ = 0.42) and tended to be effective at increasing hot carcass weight at wk 34 ($P_{Treatment}$ = 0.07; **Figure 2**). During the initial feedlot period (wk 12) no significant effect of feeding Se-enriched hay on BW was observed (+2.2%; $P_{Treatment}$ = 0.58). At slaughter, no significant differences were observed for carcass quality grade levels [prime (highest), choice, select, standard, no-roll (lowest)], as both groups had all but 4 animals with choice grade ($P_{Treatment}$ = 0.53). However, yield grade [1 (most desirable trim); 2, 3 (industry average), and 4] were improved in animals that were fed Se-enriched alfalfa hay during the preconditioning period ($P_{Treatment}$ = 0.008), as more calves of the group fed Se-enriched alfalfa hay had a 1 or 2 (86%) compared with calves in the control group (36%). Slaughter data were not available for 4 calves in the control group and 1 calf in the high-Se treatment group because ear tags were lost at slaughter.

Fecal parasite counts

We evaluated fecal parasite load at wk 0, 5, and 9 (end) of the treatment period. Overall, fecal parasite counts were low; especially counts for *Nematodirus*,

Maniezia, and *Trichuris* were mostly zero. The range for coccidia egg counts were between 0 and 600 eggs/g and for trichostrongyle-type egg counts were between 0 and 450 eggs/g. Feeding Se-fertilized alfalfa did not significantly alter coccidia ($P_{Treatment} = 0.96$) and trichostrongyle-type egg counts ($P_{Treatment} = 0.81$). No significant time nor treatment \times time interactions were observed. At the beginning of the feedlot period, calves were dewormed. As a result, fecal counts were zero or \leq 25 eggs/g feces in wk 12.

Conclusions

In summary, as we have shown previously, Se fertilization of alfalfa fields in a region with Se-deficient soils increases Se content of alfalfa hay. Feeding recently weaned beef calves with this hay resulted in increased WB-Se concentrations and improved growth rates prior to entering the feedlot. These effects tended to be associated with long-term beneficial effects on performance in the subsequent feedlot period, evidenced by a tendency for heavier hot carcass weights in calves fed the Se-enriched alfalfa hay. Because fecal trichostrongyle-type egg counts were low in all calves, group differences were not apparent, and a beneficial effect of Se supplementation on naturally acquired parasite infection in beef calves was not observed. Nonetheless, our results suggest that building Se-body reserves by feeding supranutritional Se concentrations of Se-enriched alfalfa hay during the preconditioning program is an effective management strategy to optimize growth of weaned beef calves.

Acknowledgements

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Table 1. Alfalfa and grass hay nutrient compositions (dry matter basis)^{1,2,3}

Nutrient	Alfalfa Hay		Grass Hay
	Control	High-Se	
Dry matter, g/kg	842	870	883
Crude protein, g/kg	163	170	75
Acid detergent fiber, g/kg	317	332	435
Neutral detergent fiber, g/kg	390	393	668
Nonfiber carbohydrates, g/kg	371	343	195
Fat, g/kg	0.2	<0.1	0.1
Ash, g/kg	75.8	95.1	61.9
TDN, g/kg	631	609	549
Calcium, g/kg	17.2	18.0	4.8
Phosphorus, g/kg	2.4	2.4	1.6
Magnesium, g/kg	5.3	5.4	2.6
Potassium, g/kg	9.7	10.0	6.0
Sodium, g/kg	3.9	3.8	3.2
Copper, mg/kg	14	15	6
Iron, mg/kg	627	1196	214
Manganese, mg/kg	39	56	116
Zinc, mg/kg	19	21	28
Selenium, mg/kg	0.06	3.47	0.12

¹Alfalfa and grass hay samples were submitted to Cumberland Valley Analytical Services, Waynesboro, PA for routine nutrient analysis and to Utah Veterinary Diagnostic Laboratory, Logan, UT for Se analysis.

²Alfalfa and grass hay DM determination was completed at a temperature of 105 °C for 12 to 14 h in a forced draught oven. Methods for CP, ADF, ash, and minerals were performed according to the Association of Official Analytical Chemists (2000). The NDF was determined according to Van Soest et al. (1991). Soluble protein was determined according to Krishnamoorthy et al. (1982).

³Alfalfa and grass hay samples were prepared for Se analysis as described by Davis et al. (2012), and Se determined using inductively coupled argon plasma emission spectroscopy (ICP-MS; ELAN 6000, Perkin Elmer, Shelton, CT).

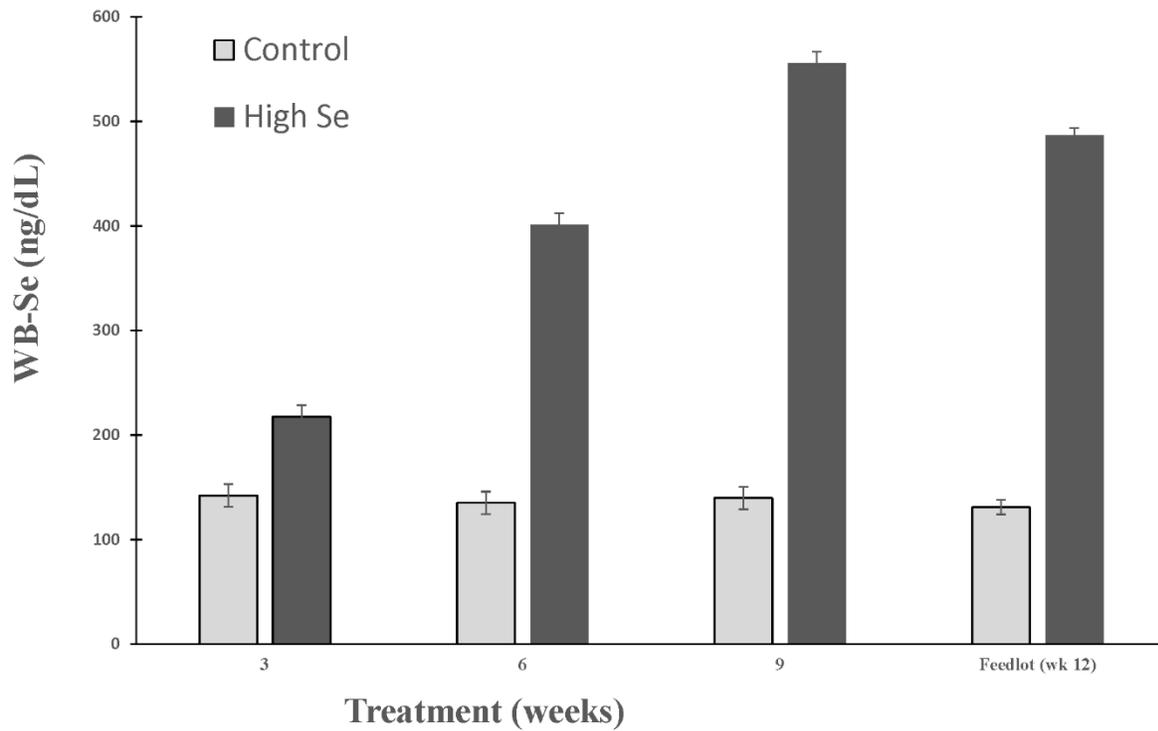


Figure 1. Comparison of whole-blood Se concentrations (mean \pm SEM) in weaned beef calves consuming alfalfa hay grown in fields not fertilized with Se (control) or harvested from a field fertilized with sodium-selenate (application rate of 89.9 g Se/ha) for 9 wk (n=15 calves per group). Total dietary Se intake during weeks 7 to 9 was 1.09 and 27.45 mg Se/calf/day, respectively for calves in control and high-Se treatment groups. The normal reference interval for whole-blood Se concentrations of beef cattle is 120 to 300 ng/mL.

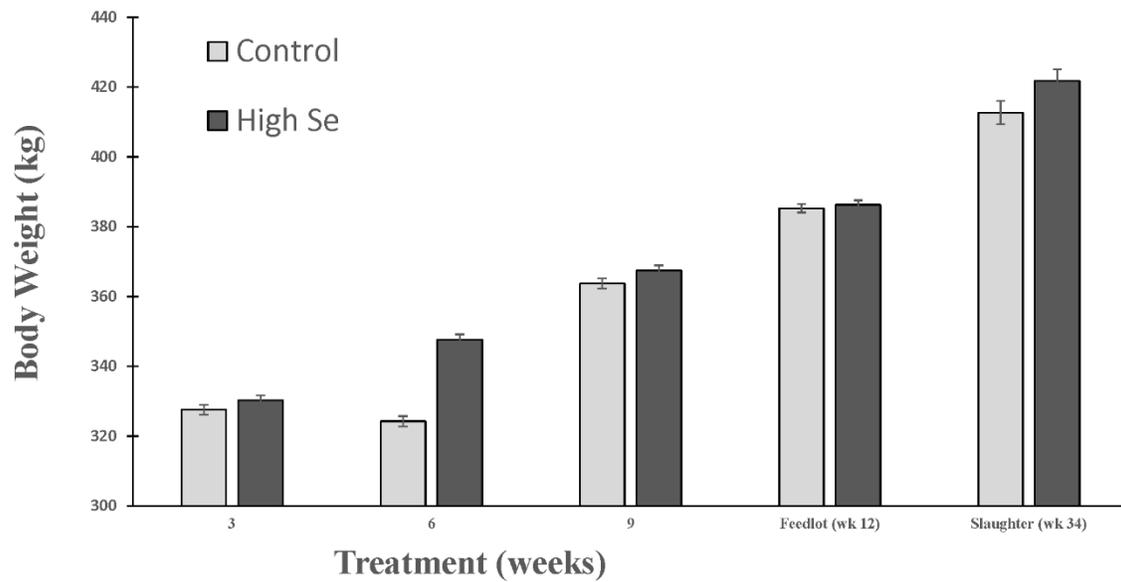
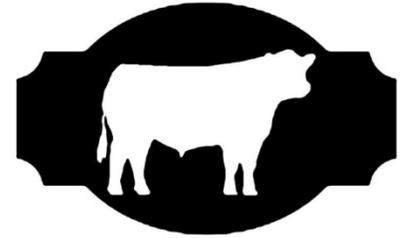


Figure 2. Comparison of baseline-adjusted BW (kg; mean \pm SEM) of weaned beef calves (primarily of Angus breeding and ranging in age from 6 to 9 months at baseline) and BW after consuming alfalfa hay grown in fields not fertilized with Se (control), or harvested from a field fertilized with sodium-selenate (application rate of 89.9 g Se/ha) for 9 wk (n=15 calves per group). Body weights at baseline ranged from 264 to 369 kg (328 ± 5 kg, mean \pm SEM), and final body weights at the end of the preconditioning period ranged from 288 to 411 kg (366 ± 5 kg, mean \pm SEM).



Oregon Beef Council Report



Beef Cattle Sciences

Intrauterine Platelet Rich Plasma Reduces Endometrial Inflammation In Postpartum Beef Heifers¹

Elizabeth Puttman,² Michelle Kutzler³

Synopsis

Intrauterine platelet rich plasma reduces cervical discharge, cervical diameter, and endometrial leukocytes counts in beef heifers when administered two weeks postpartum.

Summary

The objective of this research was to compare the effects of intrauterine administration of platelet-rich plasma (PRP), platelet-poor plasma (PPP), or saline on cervical discharge score (CDS), endometrial cytology, and endometrial bacteriology at two and four weeks postpartum in 15 heifers located at the Oregon State University Soap Creek Ranch, Corvallis, OR. We hypothesized that PRP would significantly reduce postpartum endometritis. Crossbred beef heifers were restrained in a chute for sample collection and treatment. Cervical discharge was scored between 0 (no mucus) and 5 (copious purulent mucus). Cytobrush-collected cytology slides were stained with Diff-Quik® and 200 nucleated cells were counted at 400X magnification to determine the percentage of macrophages (%MACs). Quantitative aerobic bacterial culture was performed at the Oregon State University Veterinary Diagnostic Laboratory. Results were

reported as mean±SD. A Student's t test was used to compare results from the two time points. The CDS decreased in the PRP and saline groups from 3.2±1.12 to 0.73±0.96 (p=0.011) and 2.25±0.5 to 0.75±0.96 (p=0.012). The %MACs in the PRP group decreased from 3.4±1.75% and 1.0±0.79% (p=0.0012). The total aerobic bacterial culture for all groups did not decrease significantly. Cervical diameter decreased significantly in the PRP group (from 3.57±0.30 to 3.2±0.24, p=0.02) and PPP group (from 3.66±0.53 to 3.04±0.49, p=0.04). These data support further research on the molecular mechanisms by which PRP reduces postpartum intrauterine inflammation.

Introduction

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 cattle 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 beef cattle 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 animals 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. In addition, Sheldon and colleagues (2016) reported that subclinical endometritis reduces pregnancy rates in beef heifers by 16%. Despite this, research in this area has been very limited.

In cattle, many therapeutic agents and procedures have been used to treat endometritis, including systemic or intrauterine administration of antibiotics, or administration of PGF2 α (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 score, and cervical diameter.

Materials and Methods

The platelet rich plasma was prepared by collecting blood from a donor Jersey in good health and free of contagious diseases (e.g. Johne’s disease, bovine leukemia virus), using the double centrifugation method used by Lange-Consiglio and colleagues (2014). Individual centrifuge tubes of PRP and platelet poor plasma (PPP) were frozen at -80°C , thawed at 37°C , and then combined. The PRP and PPP were subjected to aerobic and anaerobic bacteriological examination to verify their sterility. The platelet concentration of PRP was determined through use of a hemocytometer and the concentration was determined to be 1×10^9 platelets/ml. Finally, the PRP and PPP were aliquoted in 10 ml ready-to-use doses and kept frozen at -20°C until use.

Commercial crossbred Angus heifers ($n=15$) at the Oregon State University Soap Creek Ranch were used for this study. Heifers were closely monitored and allowed to calve unassisted. Only heifers with normal deliveries (e.g. no evidence of prolonged second stage labor or dystocia) were used in this study. The calf’s birth weight and gender were recorded. Two weeks after calving, the reproductive tract was transrectally imaged using a high-resolution ultrasound scanner equipped with a 7.5 MHz linear array probe (M5 Digital Ultrasound, Mindray). Longitudinal images of each cervix were digitally captured to quantify the cross-sectional diameter. Following the ultrasound exam, the perineum was cleaned and a vaginal speculum exam was performed. A post-cervical vaginal discharge score was recorded, from 0 (no mucus) to 5 (copious purulent mucus) adapted from de Boer et al (2015).

Endometrial samples were then collected using a double guarded culture swab (2 swabs per heifer) and a cytobrush. The first swab was submitted for aerobic bacteriology, to identify the most abundant species of aerobic bacteria and quantify the number of bacterial colonies. A second swab was frozen immediately and stored at -20°C for use in a future study. Endometrial cells that were collected with a cytobrush were prepared for cytology using the method described by Pascottini et al (2015). The slides were examined using 1000X magnification and oil immersion. A

minimum of 200 cells were counted on each slide, differentiating between neutrophils, macrophages and epithelial cells so the percentage of neutrophils and macrophages could be calculated.

Following sample collection, heifers were randomly allocate into three groups: PRP (n=5), PPP (n=5), or saline (n=5). In all cases, an intrauterine infusion (10 ml) was aseptically administered using a disposable sterile catheter inside a protective sheath that was guided through the cervix via transrectal manipulation. Two weeks after treatment (4 weeks after calving), transrectal ultrasonography, cervical discharge evaluation and endometrial sample collection were repeated to evaluate treatment efficacy.

Results were reported as mean \pm SD. A paired Student's t test was used to compare results from the two time points. Significance was defined as $p < 0.05$.

Results

The post-cervical discharge score decreased in PRP and saline groups ($p < 0.05$; Figure 1). In addition, cervical diameter decreased in the PRP and PPP groups (Figure 2). There was a trend for a decrease in the percentage of neutrophils in the PRP group ($p < 0.10$) with no differences in the PPP and saline groups (Figure 3). In addition, the percentage of macrophages decreased in the PRP group, but not in the PPP or saline groups (Figure 4). Both neutrophils and macrophages are white blood cells present in endometrial cytologies consistent with endometritis. There was no difference in the number of total aerobic bacteria cultured for PRP, PPP and saline groups (Figure 5).

Conclusions

This study demonstrated the efficacy of novel intrauterine treatment with PRP on decreasing the percentage of endometrial inflammatory cells (neutrophils and macrophages) in postpartum beef heifers. Additional research on the molecular mechanisms by which PRP reduces postpartum intrauterine inflammation is needed. Although this study was performed on heifers following normal calving, further investigation is needed on the effects of administering intrauterine PRP to heifers following abnormal calving (dystocia). This treatment option may provide beef producers with a lower cost, residue-free method to improve fertility.

Acknowledgements

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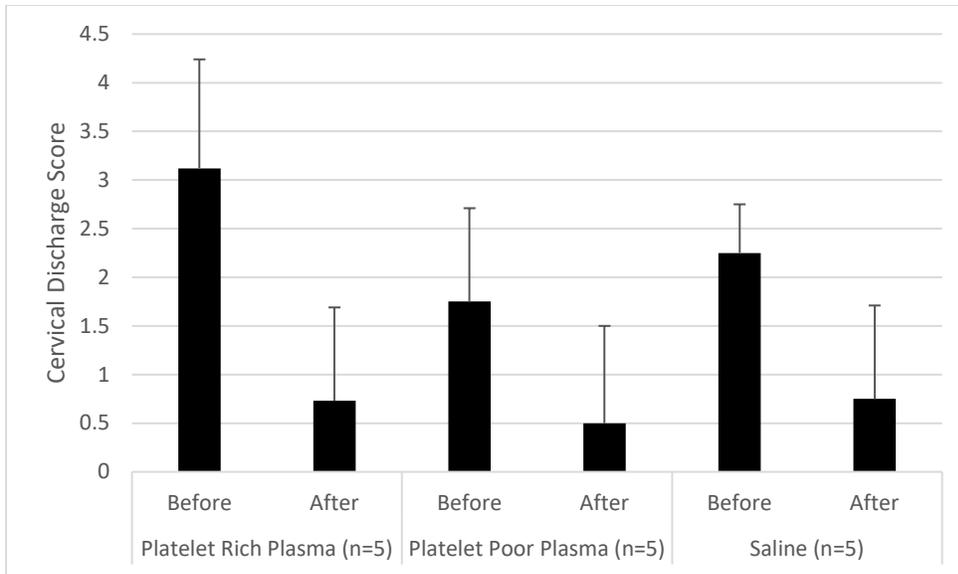


Figure 1. Cervical discharge score (mean \pm SD) as determined from vaginal speculum exam using a scoring system previously defined by de Boer et al (2015) 2 weeks (Before) and 4 weeks (After) post-calving. Heifers received a single intrauterine treatment 2 weeks post-calving. *Indicates difference ($p < 0.05$) between time points.

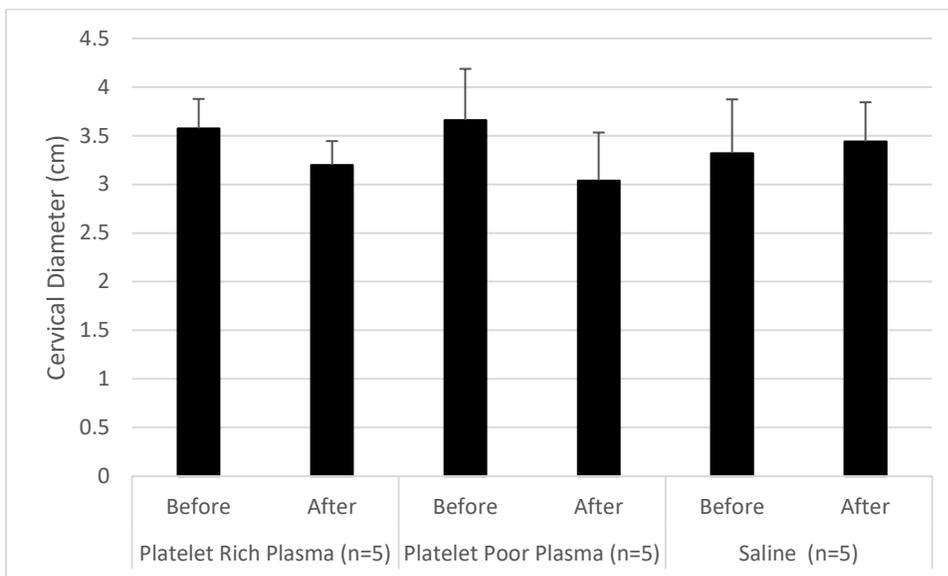


Figure 2. Cervical diameters (mean \pm SD) as measured by transrectal ultrasonography 2 weeks (Before) and 4 weeks (After) post-calving. Heifers received a single intrauterine treatment 2 weeks post-calving. *Indicates difference ($p < 0.05$) between time points.

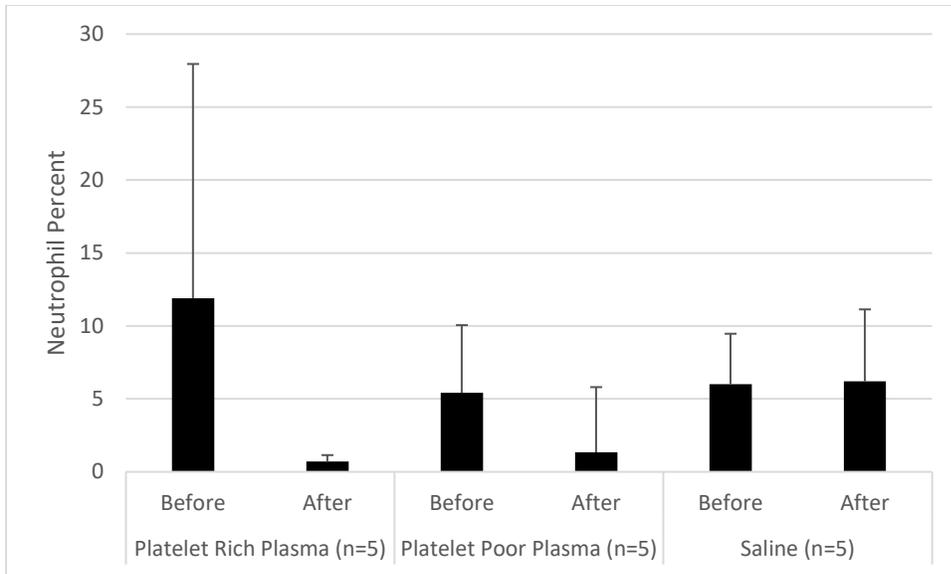


Figure 3. Neutrophil percent (mean ± SD) as calculated from cytology 2 weeks (Before) and 4 weeks (After) post-calving. Heifers received a single intrauterine treatment 2 weeks post-calving. § Indicates a trend ($p < 0.10$) for difference between time points.

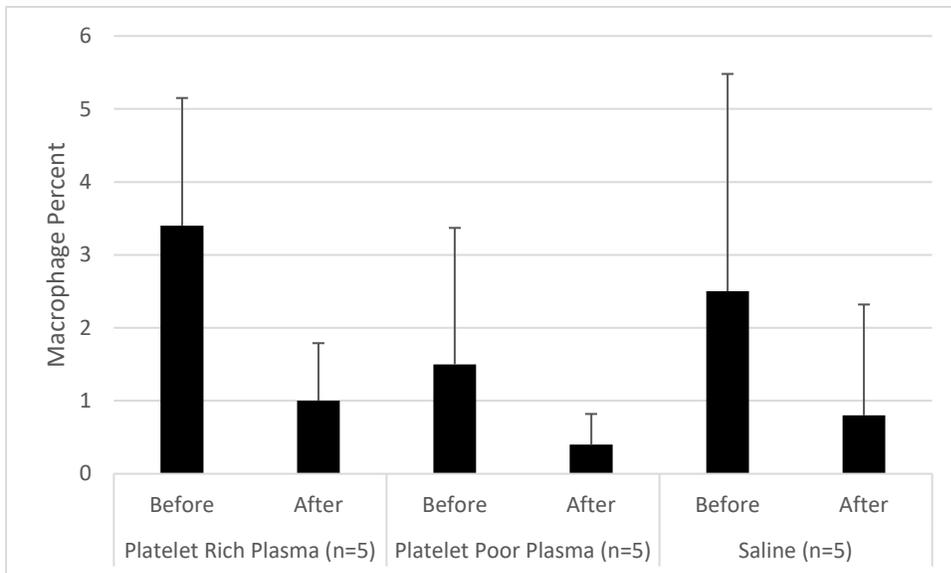


Figure 4. Macrophage percent (mean ± SD) as calculated from cytology 2 weeks (Before) and 4 weeks (After) post-calving. Heifers received a single intrauterine treatment 2 weeks post-calving. *Indicates difference ($p < 0.05$) between time points.

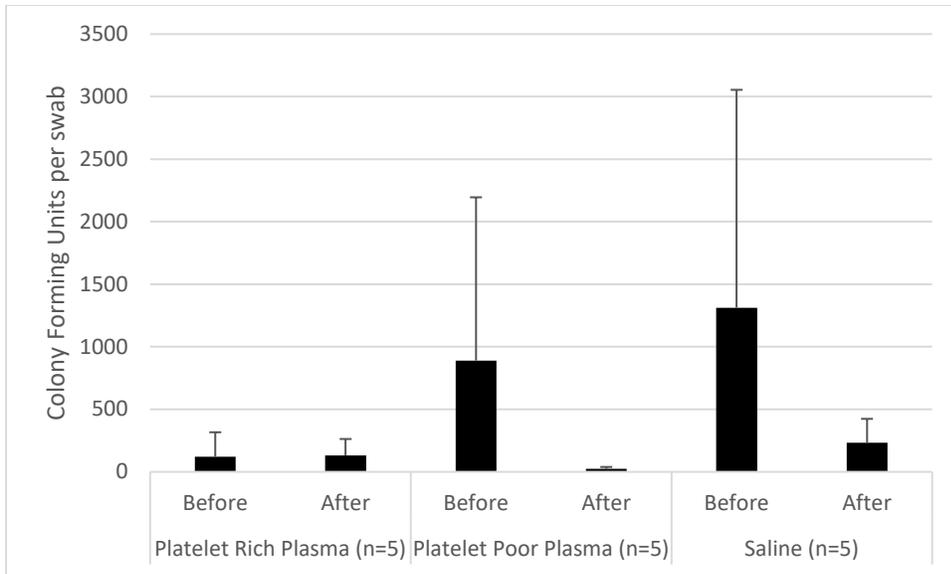
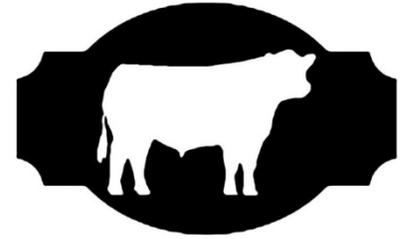


Figure 5. Total aerobic bacteria count (mean \pm SD) as measured from endometrial culture swabs at 2 weeks (Before) and 4 weeks (After) post-calving. Heifers received a single intrauterine treatment 2 weeks post-calving. There were no significant differences ($p < 0.05$) between time points.



Oregon Beef Council Report



Beef Cattle Sciences

An in vivo-in vitro hybrid system to perform nutrigenomic studies in cattle: validation using peripartum cows¹

Massimo Bionaz,² Sebastiano Busato³

Synopsis

The use of serum collected from cows and bovine cells was an effective method to demonstrate a nutrigenomic effect of free fatty acids released by the fat tissue on liver and mammary.

Summary

Dairy cows undergo a significant degree of metabolic stress in the transition between pregnancy and parturition. The resulting negative energy balance causes a breakdown of body fat, which enters the bloodstream as non-esterified fatty acids (NEFA). It is unclear if NEFA have nutrigenomic properties, but it is difficult to assess this in vivo. In order to overcome such challenge, in the present work we developed a in vivo-in vitro hybrid method. We hypothesized that high circulating NEFA in the peripartum can be used as a substrate for the activation of the Peroxisome Proliferator-Activated Receptor (PPAR), a transcription factor with known nutrigenomic effects. We collected blood from 3 jersey cows at three different points (-40d, -10d and +10d relative to parturition) and used the serum from those to treat mammary (MAC-T) and liver (BFH-12) cells to assess PPAR activation. The results showed a great response in both cell lines to increasing concentration of circulating NEFA in the blood, demonstrating the causal link between the two in the transition period. Furthermore, we

demonstrated that circulating NEFA preferentially activate PPAR δ , followed by PPAR γ and PPAR α (but not in BFH-12). By comparison, we tested the effect of palmitic acid, one of the most common fatty acids, showing that palmitic acid alone activated PPAR δ and PPAR α , but not PPAR γ . This suggests that additional fatty acids found in the NEFA pool are responsible for the activation of PPAR in the transition period. Our results substantiate the importance of acquiring further insight on the interaction between NEFA and PPAR, and highlight the viability of the in vivo-in vitro hybrid system developed and reinforce the importance of nutrigenomics as a potential tool for farmers.

Introduction

Nutrigenomics is a scientific branch of nutrition that studies how molecules contained in feedstuff can modify the biology of the organism by changing the expression of specific genes in cells (Bionaz et al., 2015). The study of nutrigenomics is very important because, once deciphered the effect on the transcription of genes of a compound present in the feed, we can use such effect to fine-tune the biology of an organism by increasing (or decreasing) the amount of that specific compound in the diet.

It is becoming evident that fatty acids (the main components of the fat in the diet) can affect the expression of genes. They can do this by binding and activating proteins (i.e., transcription factors) in cells that turn on or off specific genes. It is known that certain fatty acids, such as palmitic acid (one of the

most common fatty acid present in animal products) have a positive effect on the overall physiology of the cattle when added into the diet (Loften et al., 2014). Besides the overall physiological improvement, these fatty acids also increase the production of milk fat. This is partly due to the augmented availability of fatty acids from the diet to synthesize fat. However, recent data seem to suggest a nutrigenomic effect of dietary fat on cows, including milk fat synthesis. Mammary epithelial cells (i.e., the cells that produce milk in the mammary gland) treated *in vitro* with palmitic acid had an increased expression of genes involved in milk fat synthesis (Bionaz et al., 2013). Data indicated that the nutrigenomic effect of palmitic acid was due to the activation of a transcription factor called peroxisome proliferator-activated receptor (PPAR). This protein plays a very important role in controlling the metabolism of fat in mammals but also can help immunity and milk production.

The transition between pregnancy and parturition, especially in the early post-partum, is the most challenging period for lactating cows. The change in the overall metabolism to cope with the large amount of milk produced puts the cows in metabolic distress. One of the main problems animals have to face is the limited amount of energy from food post-partum to satisfy the large energy demand for the milk production. In order to cope with the lack of energy, the cows start to mobilize fat in the form of non-esterified fatty acids (NEFA) from the fat tissue. For this reason the concentration of NEFA in blood increases substantially just after parturition (Loor et al., 2013a). The liver plays a chief role in keeping the amount of NEFA to a non-toxic level in blood by transforming the NEFA in ketone bodies and storing it as fat; thus, the liver plays a chief role in the use of NEFA around calving. Prolonged large amount of NEFA in blood can be detrimental for the health of the cow. This metabolic situation puts the dairy cows under tremendous metabolic and oxidative stress and increases the chance of post-partum health problems, including ketosis and excessive accumulation of fat in the liver (Drackley et al., 2001). It has been proposed that the activation of PPAR in liver by NEFA can enhance the utilization of fatty acids, minimizing the negative effects of fat accumulation postpartum in the ruminant's liver (Drackley, 1999). Some indirect evidence of such hypothesis have been produced; for instance, it has been shown that cows that are feed-restricted during the dry period (especially in the close-up) and, thus, mobilize NEFA earlier in the transition period compared to cows fed a high-energy diet, have a liver able to face the metabolic challenges

during the postpartum (Shahzad et al., 2014). Recently it was proposed that the activation of PPAR using specific dietary fatty acids present in the feedstuff can help improving the post-partum situation. This proposal was based on previous data and it appears to be supported by recent data, mostly produced using *in vitro* approaches (Bionaz et al., 2013); however, a direct *in vivo* proof still need to be provided.

Circulating NEFA mostly derive from the lipomobilization of LCFA stored as triglycerides in adipose tissue. Thus, besides *de novo* fatty acid synthesis, NEFA composition is a direct consequence of dietary fat. Therefore, if the large increase of NEFA around parturition can activate PPAR, it might be possible to modulate PPAR by feeding specific fatty acids during the dry period. With the exception of indirect evidence (Bionaz et al., 2013), *the literature presents no evidence of a direct role of NEFA on bovine PPAR*. Data in monogastric animals indicate that circulating NEFA do not activate PPAR due to insufficient dose to elicit a response, even in fasting animals, while a response was observed in NEFA released locally from circulating fat transported as lipoproteins (Ruby et al., 2010). If this is true also in ruminants, important practical consequences for nutrigenomic interventions are but a logical sequitur. This underlines the importance of determining whether *the large NEFA in blood early post-partum in dairy cows can activate PPAR*, particularly in liver and mammary cells, which play a major role during the transition from pregnancy to lactation (Loor et al., 2013b). Furthermore, there are 3 types of PPAR, called PPAR α , PPAR δ , and PPAR γ with important functional difference in the body. To understand which of the types of PPAR is activated by NEFA or fatty acid is paramount.

Nutrigenomics is a very challenging field of study. Two main approaches can be used to carry out such studies. The most popular is the use of the *in vitro* approach, where bovine cells are isolated from tissues and grown in dishes in an incubator. The cells are then used to assess the nutrigenomic effect of various compounds. This approach has the advantage of being relatively cheap, easy to control, and allows high precision and replication. However, among the downsides is the fact that the cells are removed from the body, losing their ability to “communicate” with other tissues through hormones and other signaling molecules contained in the blood. The use of *in vivo* approaches (i.e., use of animals) to study of nutrigenomics, although more biologically relevant, is extremely challenging. Chiefly, the nuanced reality of

the nutrigenomic effect of dietary compounds presents a significant pitfall, as repeated sample collections over time are often too resource-intensive, invasive, or outright impossible. In addition, the complexity of the response of the animals to treatments does not allow to have the desired precision for nutrigenomic studies. Therefore, alternative methods are in high demand in order to make fundamental advances in the field of nutrigenomics.

Our goal is to develop an *in vivo-in vitro* hybrid approach to carry out nutrigenomic studies in cattle. The objective for the present work was to develop an *in vivo-in vitro* hybrid system focused on mammary tissue and liver, and test the hypothesis that NEFA present in serum of cows around parturition activate PPAR in liver and mammary cells of cattle.

Materials and Methods

Animals and sample collection

Experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Oregon State University (#4894). Cows used for the present experiment were part of a larger experiment where animals were fed Selenium-biofortified alfalfa hay or a control alfalfa hay. For the present experiment only samples for the control animals were used. Cows were pregnant Jersey heifers. Liver tissue was obtained via biopsy as previously described ([Loor et al., 2006](#)). Dr. Charles Estill performed the biopsy.

Isolation of blood serum, NEFA, and neutral lipids

Blood was obtained in evacuated tubes from 3 Jersey dairy cows at -40, -10, and 10 days relative to parturition. Serum was obtained by centrifugation at 1,500xg for 15 min and preserved at -20C until use. NEFA and neutral lipids (NL) were isolated from the serum using a previously described method ([Contreras et al., 2010](#)). NEFA and NL were resuspended in DMSO. NEFA solution was neutralized by using NaOH prior being used for the experiments. NEFA was measured in serum, NEFA, and NL using a commercial kit (Wako Diagnostics).

Isolation and cultivation of primary bovine liver cells

Isolation of primary hepatic cell was performed following several published methods ([Spotorno et](#)

[al., 2006](#), [Lemley and Wilson, 2010](#), [Panda et al., 2015](#)); ultimately, a manual perfusion technique was identified as the most efficient technique. Briefly, liver sections from performed biopsies (~300 mg) were punctured longitudinally with a 24-gauge needle attached to a 50mL syringe. The tissue was then retracted, so that only the tip of the needle was inserted in it, and it was secured using a pair of curved locking forceps. The tissue was immersed in Hank's Balanced Salt solution (without Ca⁺⁺ and Mg⁺⁺), and 50mL of HBSS + 0.05 mM EGTA were flushed through the tissue at a rate of 25 mL/min, to weaken cell junctions. Cells were then dissociated using 10 mL of a 0.1% Type IV collagenase solution in HBSS (with Ca⁺⁺ and Mg⁺⁺), at a rate of 5mL/min, repeated three times. The tissue was then mechanically disrupted using a scalpel blade, centrifuged three times, and finally filtered through a 70 µm cell strainer. Cells were resuspended in culture media after the final wash, counted and plated in different formats (including T-75 cell culture flasks 6- and 12-well plates polypropylene cell culture plates, with and without Matrigel coating). Cells were cultured in William's E medium supplemented with FBS, insulin, glucagon and dexamethasone for up to four days, or until complete apoptosis was detectable.

Culture of liver tissue

Liver tissue core from performed biopsies were finely sliced using a Krumdieck Tissue Slicer (Alabama Research, USA). Briefly, liver cores were embedded in 3% low-temperature agarose to account for size differences between the biopsy probes and the available coring tool from the manufacturer. Several slices were obtained, all with measurable thickness of ~300 µm. Precision-Cut Liver Slices (PCLS) were visually judged and uneven or imperfect slices were discarded or frozen for further analysis. Suitable PCLS were incubated in 12-well polystyrene plates, and exposed to either control (William's E medium + GlutaMAX and insulin) or treatments. Cells were incubated in a carbogen environment (95% O₂, 5% CO₂), ensured by maintaining the culture plates in a flushed Billups-Rothenberg hyperoxia chamber, for 24 hours. Constant rotation (270 rpm) was maintained during the entire incubation. At the end of each period, PCLS were patted dry and weighed, and their RNA was isolated by Phenol-Chloroform separation, followed by magnetic retrieval using MagMAX 96 Total RNA (Thermo Fisher Scientific, USA)

Isolation and culture of bovine primary mammary cells

Mammary cells were isolated as previously described ([Danowski et al., 2013](#)) with several modification. Semi-sterile milk collection was achieved using a 1000 mL Büchner flask with a silicone teat cup liner fitted to the wide mouth of the flask. The vacuum line originally supplying the claw was connected to the flask, effectively shielding the sample from external contaminants. The teat was also thoroughly cleaned with water, iodine solution and ethanol. Samples were immediately transferred to the lab, where the milk fat was separated by centrifugation and washed three times in HBSS supplied with high concentrations of antibiotics and antimycotics. Finally, cells were plated in T-75 culture flasks and cultured in DMEM with a cocktail of growth-promoting hormones.

Culture, Transfection and Measurement of PPAR activation in immortalized bovine cells

Mammary alveolar cells transformed (MACT) already present in our laboratory and immortalized bovine hepatic cell line (**BFH-12**) obtained from another laboratory ([Gleich et al., 2016](#)) 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). For the experiments, approximately 30,000 or 3,000 cells/well were plated in 96-well or 384-well plate, respectively. Twenty four hours later the cells were co-transfected with a PPAR Response Element associated with luciferase and a renilla plasmid at 50:1 ratio of luciferase/renilla plasmid. Treatments were applied 16 or 24 h post-transfection. Luciferase and renilla activity were measured *via* luminometer. For the experiments, also a HP 300e Digital Dispenser was used.

Treatments

In order to assess if NEFA present in the serum activate PPAR, cells were treated with serum containing low (0.2 mM - prepartum) or high (1 mM – early postpartum) NEFA or purified NEFA at the same concentration as in the sera. To assess if NEFA released from lipoproteins (i.e., from the dietary fat) we used a commercially available lipoprotein lipase isolated from bovine milk (Qiagen) at a dose of 2 U/mL, either with serum or with NL (mostly triglycerides present in lipoproteins).

Cell viability

Viability of cells was assessed using Trypan Blue exclusion or the NucBlue Live ReadyProbes Reagent (Thermo Fisher Scientific) in association with DAPI (to stain the cell nuclei)

Statistical Analysis

In vitro experiments were analyzed using a generalized linear model (proc GLM, SAS 9.4) with LSD contrasts. Several linear models were tested to analyze the effect of Serum NEFA concentration on PPAR activation, using R^2 as an indicator of goodness of fit. The statistical analysis for the latter was performed in R (3.5.1).

Results

Isolation of primary hepatocytes from adult cows.

We attempted to isolate viable hepatocytes from the liver tissues obtained via biopsy using several approaches as described in Materials and Methods. We were able to obtain pure hepatocytes but the cells quickly died and we unable to conduct any experiment. Hepatocytes from adult cows are very difficult to obtain. Prior report indicated that it is possible to obtain hepatocytes from tissue biopsy obtained from adult liver in human ([Kim et al., 1995](#)) but also in cows ([Spotorno et al., 2006](#)). However, the use of the last approach did not provide hepatocytes but rather fibroblast-like cells (i.e., non-hepatocytes). Viable hepatocytes were obtained by using the method of perfusion of the entire liver ([Elgendy et al., 2017](#)). Even with that approach cells were viable only for relatively few hours. Additionally, even if this method were to provide more reliable results, it would be inapplicable to our experiment: proper perfusion requires a large vein to deliver the solutions through the tissue, a requirement that cells for at least an entire liver lobe, and not a small biopsy core.

Use of tissue liver slices culture to study nutrigenomics effect of NEFA.

Because of the impossibility of obtaining viable hepatocytes from the liver biopsy, we decided to use the well-established liver tissue culture system. Tissue slices were cultivated in 12 well plates.

Viability of the tissue was assessed by measuring the uptake of NEFA and the activity of the hepatic lipase (i.e., an enzyme that release NEFA from circulating lipoproteins). Liver slices significantly took up NEFA when added as purified into medium; however, the amount of NEFA tended to increase after 24h of cultivation with serum, indicating a strong activity of the hepatic lipase (**Figure 2**). Once we determined that the tissue slices were viable for at the least 24h, we treated the tissues in duplicates with serum from the same cow, NEFA purified from the serum of the same cow, or serum + lipoprotein lipase, NL+lipoprotein lipase, an a synthetic agonist of PPAR α , or control (only medium). Treatments were applied for 6, 12, or 24h. A sample was also collected prior any treatment (i.e., time 0). With the intent to measure the activation of PPAR by measuring the expression of known target genes, we isolated RNA from the tissues. We tested 4 different methods, including the classical Trizol, RNaeasy column (Qiagen), DirectZol (Zymo Research), and MagMax (Fisher Scientific). By measuring RNA integrity via TapeStation 4200 or Bioanalyzer 2100 (both from Agilent), we determined that the use of MagMax provided the best quality RNA. We are now in the process of measuring the expression of PPAR target genes.

Transfection of mammary epithelial cells

Isolated mammary epithelial cells were transfected using several commercially available methods (Lipofectamine 3000, TransfeX, Effectene) and via conjugated lysine-polyethyleneimine (PEI); however, plasmid delivery in all instances has been subpar (<2%), and we are currently investigating further, more suitable methods. As a partial replacement, we utilized an immortalized model of mammary alveolar cells (MAC-T).

NEFA activate PPAR in bovine cells.

PPAR activation by NEFA was initially assessed in immortalized mammary alveolar cells (MAC-T) as their flexibility and resistance to varying experimental conditions makes them a valid preliminary model. Serum activated PPAR by ~3 fold, with the post-partum sample (high NEFA, 1.0 mM), showing greater activation than the low NEFA (0.2 mM) (**Figure 3A**). These effects were recapitulated by the isolated NEFA from the serum obtained from post-partum cows, and were further reduced nearly halfway when the sample was diluted

in culture medium at a 2:1 ratio. Interestingly, while NL did not activate PPAR (as expected), addition of LPL caused a great increase of the reporter activity, which suggest that locally released NEFA activate PPAR by a significant degree. To verify our assumption that greater circulating NEFA would induce greater PPAR activation, we designed an experiment in which we treated MAC-T cells with sera having different NEFA concentration (**Figure 3B**). The model shows a significant activation of PPAR by serum NEFA, which begins at 0.6 mM and increases quadratically, plateauing at around 1 mM. This is highly relevant, as cows routinely reach over 0.6 mM in their circulating NEFA peak during the early postpartum.

Determining PPAR activation through NEFA in the transition period is only half the battle; in order to hypothesize plausible consequences of said activation, we need to determine which PPAR isotypes are most affected by this newfound phenomenon. To obtain this key piece of information, we envisioned a series of experiment in which we treated the cells with serum combined with PPAR-specific inhibitors (GW6471 for PPAR α , GSK3787 for PPAR δ , and GW9662 for PPAR γ). The results (**Figure 3C**) show that in MAC-T cells, PPAR δ is responsible for most of the detected PPAR activity, followed by PPAR γ and finally PPAR α . Our immortalized bovine fetal liver model (BFH-12) displays similar activation pattern, with the exception of PPAR α , which does not appear to be activated at all (Figure 3D).

The results were thoroughly compared with the activation pattern displayed by one of the most detectable fatty acids in bovines, palmitic acids. Palmitic acid is unable to activate PPAR when added on the serum at relatively low dose in culture, and does not change which PPAR isotypes are activated (**Figure 4**); however, when the cells are treated with palmitate alone, significant activation is displayed, which can be attributed to PPAR δ and PPAR α , but not PPAR γ (**Figure 4**). This is a significant departure from our previous results, and suggest that palmitate alone cannot explain PPAR activation patterns in the transition period, unveiling the enticing opportunity of identifying new key players in the nutrigenomic landscape of the peripartum.

Conclusions

Metabolic stress in livestock animals is an inevitable event; yet, in a deeper understanding of the

animal's biology lies the toolkit we can capitalize on to render it more manageable and reduce economical losses. Nutrigenomic approaches to dairy management, novel in their essence, provide an opportunity to improve animal health and welfare in the early postpartum, improving long-term productivity and profitability. This study demonstrates for the first time the clear link between high circulating NEFA and PPAR activation, which so far had only been vaguely suggested. Our hybrid model, combined with the most sophisticated, state-of-the-art molecular techniques, will unveil further nutrigenomic landscapes and interactions of NEFA and dietary fatty acids with PPAR, and will take our results one step closer to practical implementations in the future.

Acknowledgements

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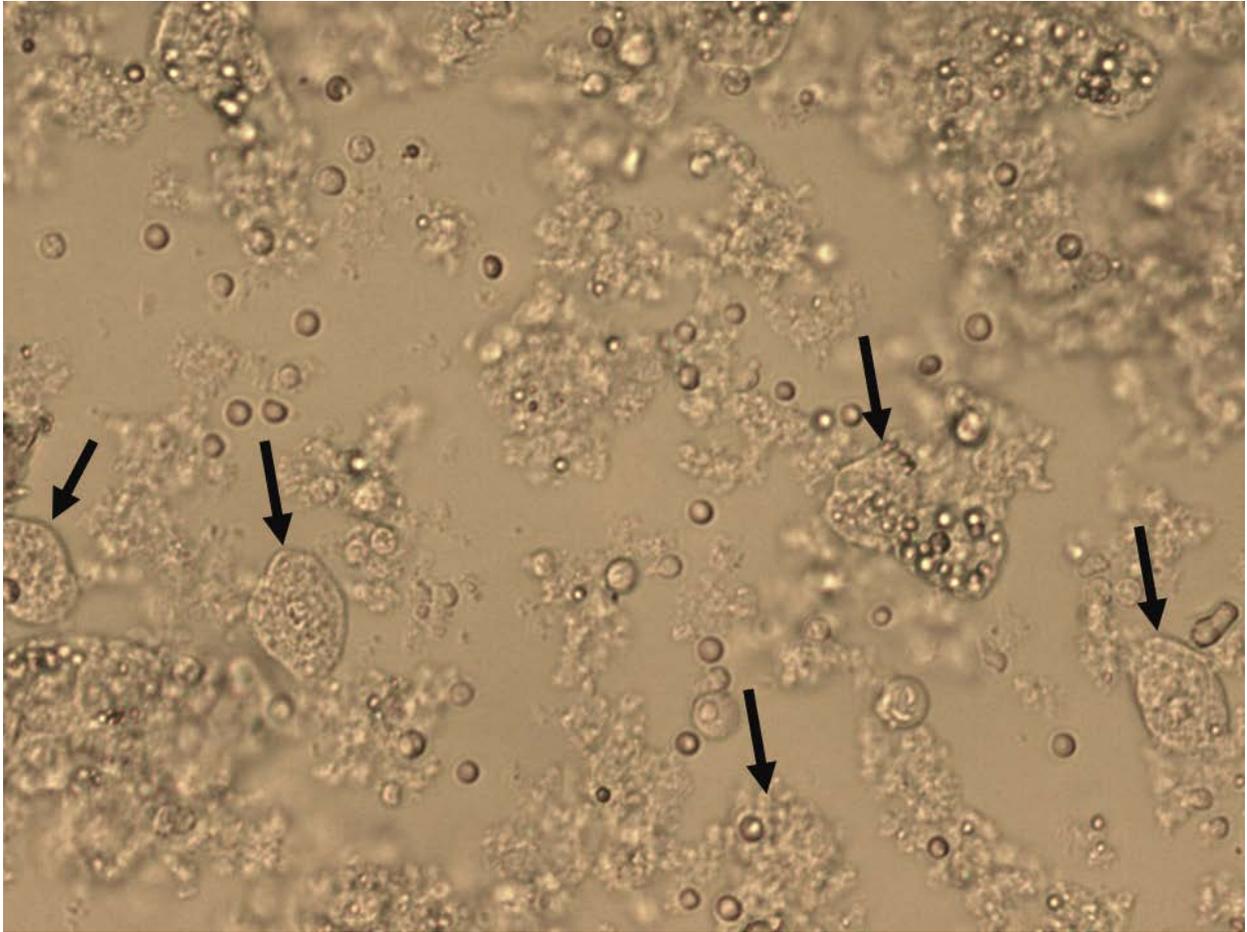


Figure 1. Hepatocytes obtained from the liver of adult cows obtained via biopsy and cultivated in vitro (black arrows). Visible inside cells are small corpuscles, likely apoptotic particle (i.e., cells were dying).

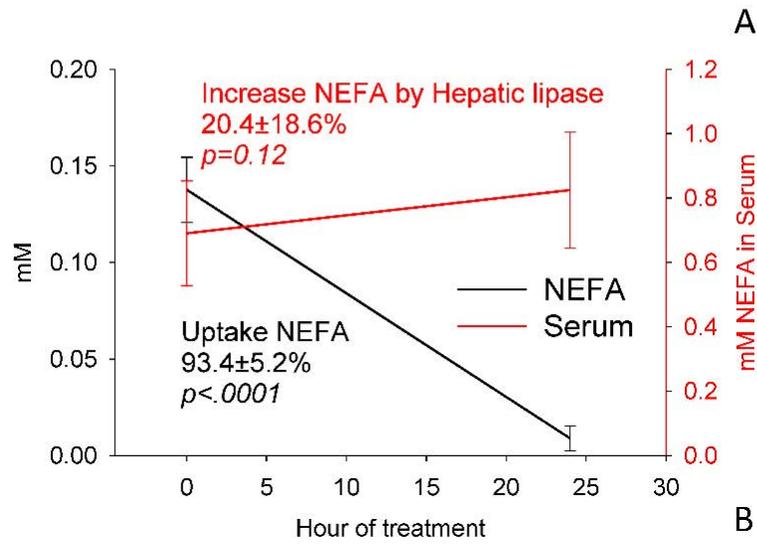
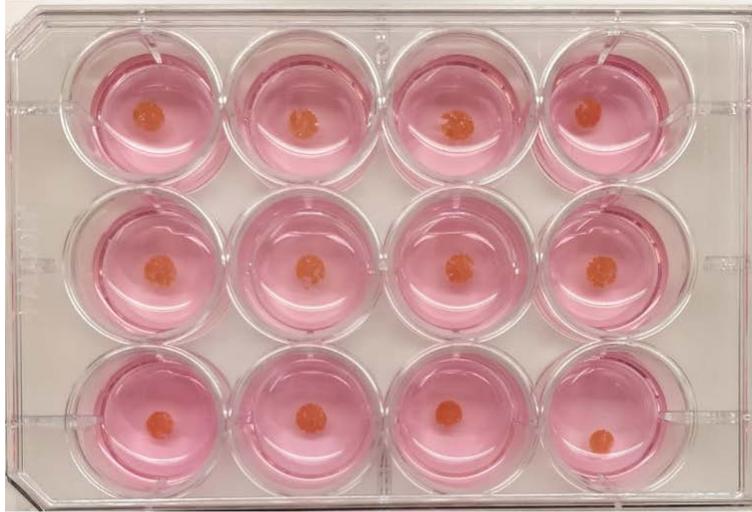


Figure 2. A. Liver tissue slices obtained from Jersey heifers and cultivated in medium ready for treatments. **B.** Liver tissue slices significantly absorbed NEFA from the media (black line). The liver tissue slices instead appear to have released NEFA from the lipoproteins present in the serum (red line).

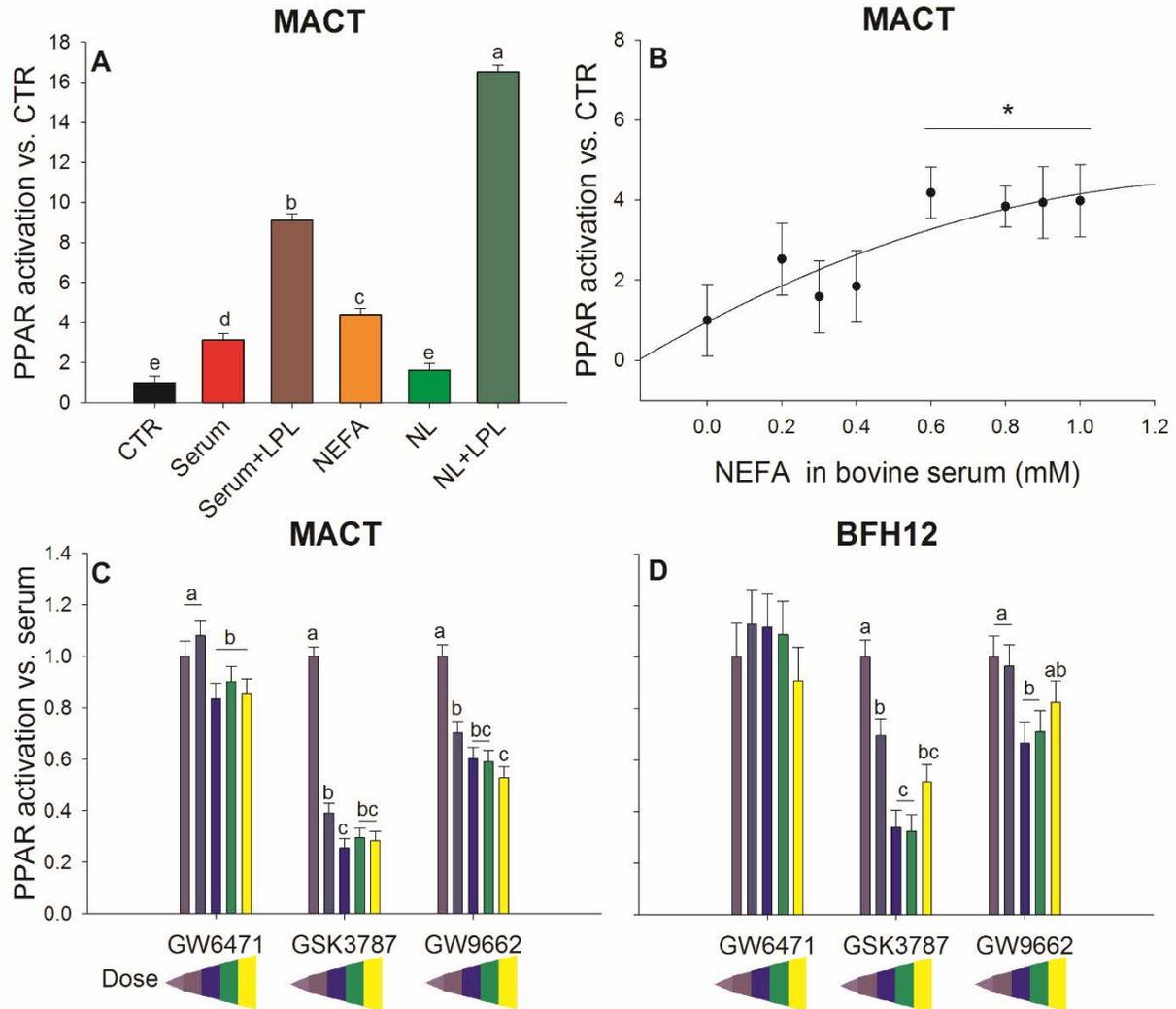
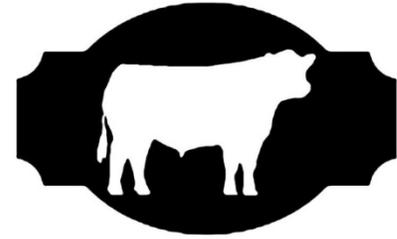


Figure 3. **A.** Bovine mammary epithelial cells (MACT) cells were treated with bovine serum, circulating free fatty acid (NEFA) isolated from bovine serum, neutral lipids (NL) isolated from bovine serum, and serum or NL treated with lipoprotein lipase (LPL) to free the fatty acids. **B.** MACT cells were treated with serum isolated from bovine blood with increased amount of NEFA. **C.** MACT cells and **D.** bovine foetal liver cells (BFH12) cultivated in bovine blood serum were treated with increased amount of PPAR inhibitors (from 10 nM to 10 μM of GW6471 as inhibitor of PPAR α , GSK3787 as inhibitor of PPAR β/δ , and GW9662 as inhibitor of PPAR γ). Different letters or * denote statistical differences.

Oregon Beef Council

Report



Beef Cattle Sciences

***Anabaena/Dolichospermum* as the source of lethal microcystin levels responsible for a large cattle toxicosis event¹**

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Synopsis

The death of 23 steers near Lakeview, OR in June 2017 was due to poisoning by microcystin toxin produced by the newly recognized *Anabaena* sp. JUN03 cyanobacterium.

Summary

Thirty-two 14-month old steers died during a period of four days (19-23 June, 2017) after drinking from Junipers Reservoir (southeastern Oregon, USA) during a cyanobacterial bloom. Microcystin-LR was present at 3000 µg/L in a reservoir water sample and at 7100 µg/L in the rumen contents of one of the mortalities. Serum biochemistry and histological examination indicated severe liver damage consistent with microcystin toxicosis. Microscopic observation of reservoir water samples was limited because samples were frozen or deteriorated, but evidence of abundant *Anabaena/Dolichospermum* was present. Genetic (metagenomic) analysis indicated the presence of a single cyanobacterium in these samples, belonging to the *Anabaena/Dolichospermum* genus and containing a complete *mcy* cluster of genes responsible for microcystin toxin biosynthesis. These results

emphasize the capacity for *Anabaena/Dolichospermum* blooms to produce lethal levels of microcystin, posing a danger to public health and livestock.

Introduction

Toxins originating from freshwater cyanobacterial blooms represent a widespread risk to humans, pets and livestock (Backer et al., 2015; Chorus and Bartram, 1999). They are of concern for livestock in both farm and rangeland settings, with toxicosis episodes being reported in many parts of the world (Briand et al., 2003); farm extension bulletins commonly raise a general awareness of the threats of cyanotoxicosis. Livestock cases in the U.S. have most frequently involved poisoning by the hepatotoxin microcystin, although all cyanotoxins are of concern. The microcystins form a group of over 100 variants that are synthesized by a c. 50-kbp *mcy* gene cluster (Dittmann et al., 2012). Microcystins have been detected in one-third to one-half of lakes sampled in the USA (Graham et al., 2010; Loftin et al., 2016). While a range of genera contain members capable of producing microcystin, *Microcystis* and *Planktothrix* are generally considered to be the most potent sources, and are often associated with high levels of the toxin (Chorus, 2012; Rinta-Kanto et al., 2009; Bozarth et al., 2010; Jacoby and Kann, 2007).

Anabaena/Dolichospermum, a genus that is also known to contain members capable of producing microcystins (Li et al., 2016), has been considered as a potential microcystin producer in risk assessments (Chorus and Bartram, 1999), but observations linking high levels of microcystin to *Anabaena/Dolichospermum* in the U.S. have been lacking. We document an event from southern Oregon in which 32 steers died after ingesting lake water containing an *Anabaena/Dolichospermum* bloom, and present evidence that the high level of microcystin-LR produced by *Anabaena* sp. JUN03 was the cause of death. Our observations show that *Anabaena/Dolichospermum* present in the U.S. can produce potent levels of microcystin.

Materials and Methods

Cattle deaths occurred at Junipers Reservoir (~30 ha) near Lakeview, Oregon, GPS coordinates 42.193647, -120.527233, beginning on 19 June, 2017. The animals were 14 month-old Angus cross steers of about 340 kg body weight. Blood and organ samples were collected during field necropsies conducted on 19 June and 21 June, and submitted for blood chemistry and histopathological analysis (Oregon Veterinary Diagnostic Laboratory, Oregon State University).

On 20 June, 2017, samples were taken from three farm drinking water sources, including Junipers Reservoir (sample JUN03), and from the rumen of a recently deceased steer. The samples were frozen and sent for microcystin analysis (California Animal Health and Food Safety Laboratory, University of California-Davis). Aliquots of these samples were sent (frozen) to Oregon State University for genetic analysis. Another sample (JUN01) containing scum was taken from Junipers Reservoir on 21 June 2017 and mailed on ice to Oregon State University for genetic analysis; this sample was detained in transit for four days, exposing it to elevated temperatures.

To conduct histopathological analysis, a selection of tissues from two steers that had been fixed in 10% neutral buffered formalin were processed and embedded in paraffin. Six μm -thick tissue sections were cut and stained with hematoxylin and eosin for examination by light microscopy.

Water and rumen samples were analyzed for microcystins -LA, -LR, -RR and -YR by LC-MS/MS (Sciex 4000 QTrap triple quadrupole /ion trap mass spectrometer). Samples were sonicated, followed by five freeze/thaw cycles to release toxins from cells. After filtration, toxins were concentrated using solid

phase extraction columns, evaporated to dryness and redissolved in methanol/water.

DNA was prepared by cell lysis with lysozyme, proteinase K and sodium dodecylsulfate followed by phenol/chloroform extraction (Bozarth et al., 2010) and purification using a DNeasy PowerBiofilm DNA isolation kit (Qiagen). DNA extracted from JUN01 and JUN03 samples was used for metagenome sequencing on an Illumina HiSeq 3000 instrument at the Center for Genome Research and Biocomputing at Oregon State University. Sequencing reads were quality screened as previously described (Otten et al., 2016) and mapped to reference sequences. PCR was also used to verify the presence of microcystin-producing *Anabaena/Dolichospermum* in the rumen sample.

Results

Thirty-two cattle died over a 4-day period near Lakeview, OR, beginning on 19 June, 2017, a hot day of 97°F. A northerly wind had concentrated a cyanobacterial bloom to form scum at the southern end of Junipers Reservoir, which served as one of the water sources available to a herd of 207 14-month-old steers. The most rapidly dying animals (19 June) showed signs of excitation, head tremors and staggering gait that progressed very rapidly to tetany and death, with or without convulsions. Most acute signs appeared to be neurological. Surviving cattle were moved to another water source on 19 June. Daily deaths were recorded as six on 19 June, 11 on 20 June, 11 on 21 June, one on 22 June, and an additional three by 23 June, totaling 32. Two recently deceased animals were necropsied in the field on 19 June and 21 June, revealing pale livers (and spleen in one animal) as the only abnormalities.

High levels of microcystin-LR were detected in the JUN03 Junipers Reservoir water sample (3000 $\mu\text{g/L}$) and in the rumen sample (7100 $\mu\text{g/L}$)(Table 1A). The WHO-recommended guideline value of 1 $\mu\text{g/L}$ is commonly used for assessing drinking water safety (WHO, 2003). A biochemical profile performed on serum collected (19 June) from a steer just prior to death on the first day of the toxicosis event revealed several values strongly indicative of hepatic damage (Table 1B). Histological examination of tissues from two animals revealed massive hepatic necrosis and hemorrhage in one animal, and extensive hepatic autolysis in the other. The findings are all consistent with microcystin toxicosis.

The samples of scum-containing water obtained from Junipers Reservoir provided limited opportunity for analysis by microscopy because they were either frozen or extensively deteriorated. Nevertheless, abundant green oblong cells interpreted as akinetes (spore-like cells) diagnostic of the filamentous Nostocales cyanobacteria were present, together with some clumps of filaments indicative of Nostocales (Fig. 1).

Metagenomic analyses of these samples revealed the presence of cyanobacterial sequences similar to the genome of *Anabaena* sp. 90 and its region containing the *mcyHIFEJDGABC* genes for microcystin synthesis (Wang et al., 2012). PCR confirmed the presence of these genes in the rumen sample. Only one cyanobacterial genome was detected, and no cyanotoxin biosynthetic genes other than the *Anabaena* sp 90-like *mcy* genes were found. The sequences of *cpcBA*, *rbcL* and *rpoB* genes showed close homology (~99% nucleotide identity) to *Anabaena* sp. 90 (Table 2). The nucleotide sequences of the *mcy*, *cpcBA*, *rbcL* and *rpoB* genes have been deposited in GenBank under the organism name *Anabaena* sp. JUN03 (Accession numbers MH663497-500).

Conclusions

Toxicology, histopathology and genetic studies presented here demonstrate that the deaths of 32 steers over a few days in June 2017 in southeastern Oregon were the result of acute liver poisoning by microcystin-LR produced by a single strain of *Anabaena/Dolichospermum*, which we refer to as *Anabaena* sp. JUN03. This cyanobacterium is closely related to *Anabaena* sp. 90, a microcystin producer isolated in Finland (Sivonen et al., 1992). Our results indicate the presence of a cyanobacterium previously unrecognized in SE Oregon that is capable of producing microcystin at dangerously high levels, threatening livestock and humans. Previous occurrences of microcystin in southern Oregon have been linked to cyanobacteria of the genus *Microcystis*. Ranchers and extension agents should be aware of the dangers posed by toxic cyanobacterial blooms, and livestock should not have direct access to lakes and streams, which can put livestock at direct risk of toxic blooms as well as adding nutrients that support the growth of blooms in lakes.

Acknowledgements

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Fig. 1. Microscopy of scum-containing water sample from Junipers Reservoir, showing akinetes (dark oblongs) and filaments indicative of *Anabaena/Dolichospermum* (*Nostocales*) cyanobacteria.

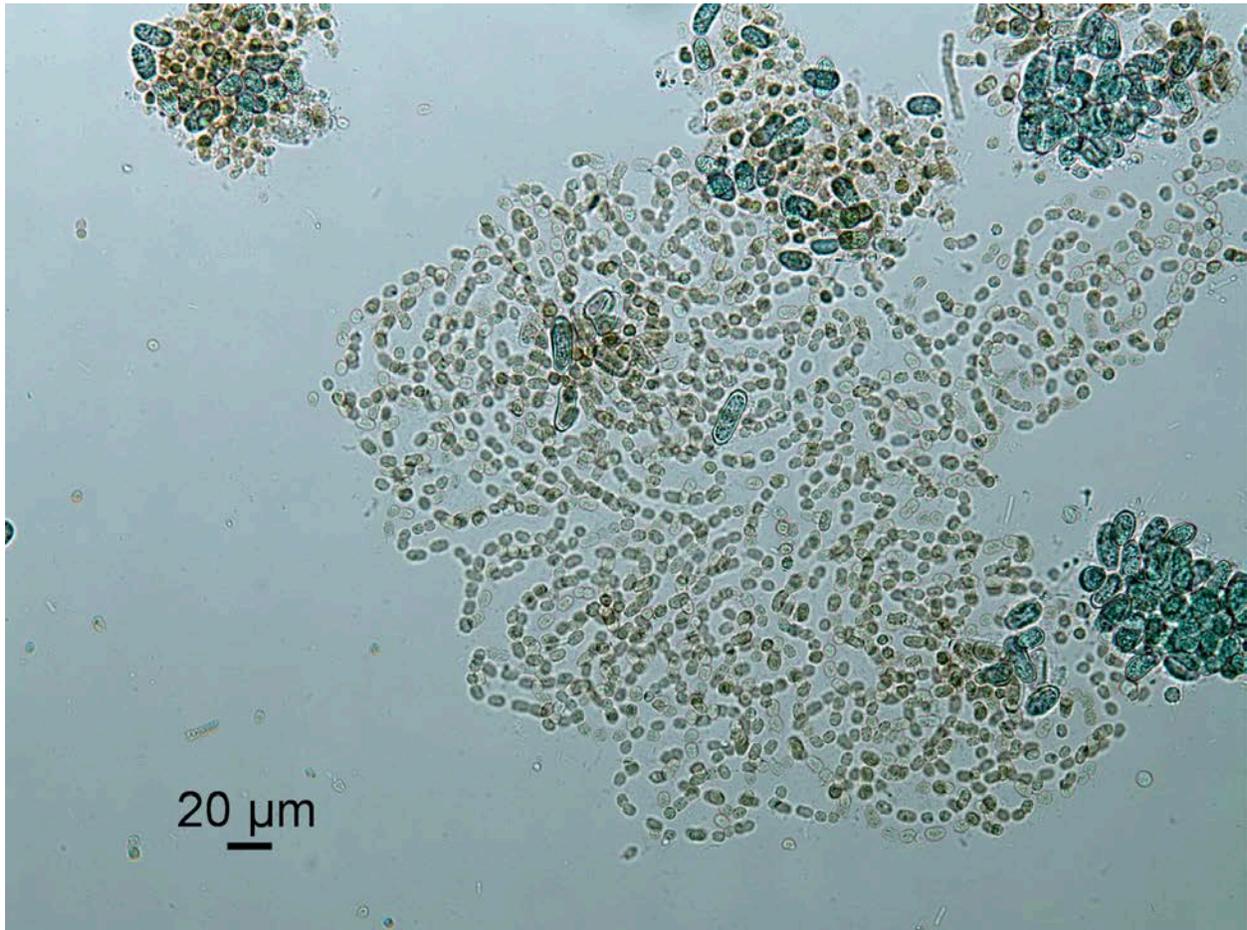


Table 1 Cyanotoxin levels in lake and rumen samples (A) and values for serum markers of liver damage in serum of acutely poisoned steer (B).

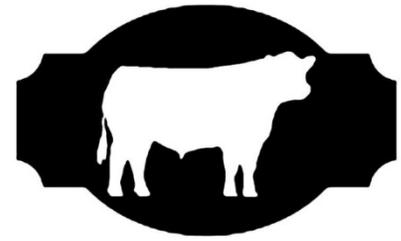
A.	Microcystin-LR concentration (µg/L)	
Junipers Reservoir (JUN03)	3000	
Rumen contents	7100	
B.		
	Concentration in steer serum	Normal concentration range
Aspartate aminotransferase	>5000 U/L	51 - 114 U/L
Sorbitol dehydrogenase	>170 U/L	0 – 50 U/L
Gamma glutamyl transferase	100 U/L	1 - 31 U/L
Alkaline phosphatase	1102 U/L	30 – 190 U/L
Total bilirubin	2.8 mg/dL	0.1 – 0.5 mg/dL

Table 2 Genes identified by read mapping to homologous genes from *Anabaena* sp. 90.

Gene(s)	Nucleotide identity to <i>Anabaena</i> sp. 90 homologs (%)	GenBank accession No.²
<i>mcyHIFEJDGABC</i>	98.92	MH663498
Other toxins		
<i>cpcBA</i> ¹	99.54	MH663497
<i>rbcL</i>	99.44	MH663499
<i>rpoB</i>	98.98	MH663500

¹*cpcBA* includes *cpcB* and *cpcA* coding regions and intergenic region

²Sequences appear in GenBank under the organism name *Anabaena* sp. JUN03.



Oregon Beef Council Report

Progress Reports – Animal Sciences ¹

Genomic Testing for Production and Performance Traits in Crossbreed Angus Cattle

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Project Objectives: Selection of replacement heifers is one of the most challenging aspects of commercial cow-calf production. Maintaining or purchasing replacement heifers is the most expensive annual cost. Females of breeding age account for about 70% of the costs of the beef cattle production system. Producers must decide whether a given heifer can be a productive cow before she has had an opportunity to express her growth and mature size, fertility, and milking ability. The significance of this decision spans all phases of the beef production supply chain. The average commercial cow has produced and weaned more than four calves before returning a profit. This makes it critical for producers to select and develop the right replacement heifers.

DNA testing may be useful for making heifer replacement decisions. The cost-benefit of DNA testing to a producer depends upon the DNA test used. There are several different types of DNA tests, including parentage tests, single gene tests (e.g. coat color, inherited defects/diseases) and single-nucleotide polymorphism (SNP) chip tests. SNP chips allow for genomic testing that may be used to optimize beneficial traits that result from multiple genes (Snelling et al 2012). These traits can be categorized on the basis of performance (e.g. average daily gain, docility, ribeye area, fat thickness, marbling) and production (birth weight, calving ease, reproductive tract score, number of antral follicles, conception rate, rebreeding rate).

Between 2015 and 2016, genomic testing in Angus seedstock increased by 45% (<http://www.angus.org/pub/annualreport2016.pdf>) and genomic testing companies (Neogen and Zoetis) are reporting that more than 1 million cattle in the United States have been tested. These companies claim accumulative value returns for genetic selection of replacement heifers. However, there have been no peer-reviewed publications validating the accuracy of the results of genomic testing on the actual traits expressed by individual animals.

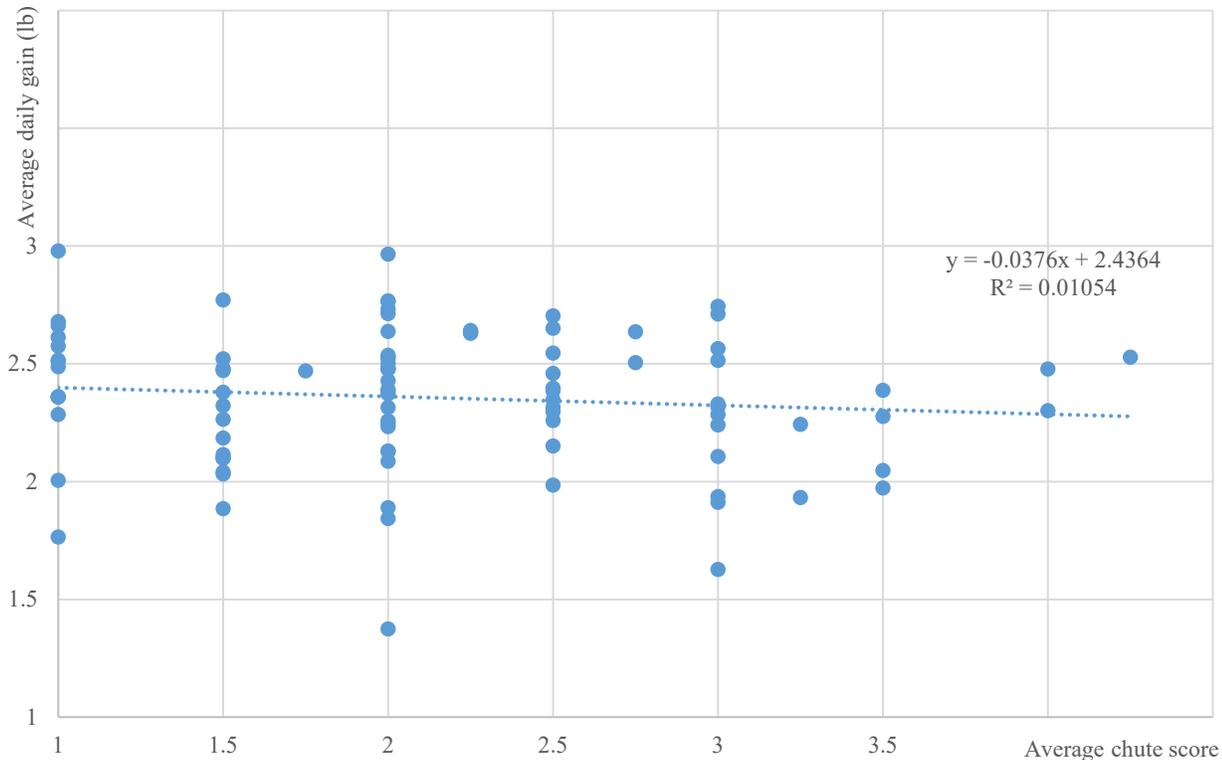
Therefore, the objectives of this proposal are to: 1) evaluate performance and production traits on crossbred beef cattle and then 2) compare these traits to the results of genomic testing. We expect that the results of this research will provide unbiased and tested information to support the decision-making process regarding the cost-benefits of genomic testing.

Project Start Date: October 4, 2018

Project Completion Date: October 2, 2020

Project status: Approximately 120 crossbred Angus cows calve annually at the Oregon State University Soap Creek Ranch. Cows are maintained on grass throughout the year with supplemental hay fed during winter months. Of these, multiparous cows delivering singleton calves (n=94) were used for this research. Weight was recorded at birth and at two days after weaning for each calf to determine average daily gain.

Selecting for docile beef cattle is not only safer for both the animals and the handlers, but docile calves have higher weaning weights than more aggressive calves (<http://www.beefmagazine.com/cowcalfweekly/04-29-docility-pays>). The Beef Improvement Federation Guidelines recommends using a chute score to measure docility. Chute scores range from 1 to 6. An animal scored as a 1 will have a mild disposition, will handle quietly, and will exit the chute calmly. An animal scored as a 2 will be somewhat restless in the chute but will be quieter than average. The animal may be stubborn during processing, with some tail flicking, and will exit the chute promptly. An animal scored as a 3, which is average, will be manageable but impatient; the animal will continuously push and pull on the head gate and will exit the chute briskly. A 4 will be flighty and slightly wild and will be jumpy and struggle violently in the chute, with continuous tail flicking, and will exit the chute wildly. An animal with a score of 5 will resemble one scored as a 4 but with increased aggressive behavior, including extreme agitation and continuous movement that may involve jumping and bellowing while in the chute. An animal rated a 5 will also exit the chute frantically and may exhibit attack behavior when handled alone. A score of 6 indicates an animal that is extremely aggressive with pronounced attack behavior. For the current research, chute scores were assessed on two consecutive days beginning one day after weaning (October 4-5, 2018) by the same evaluator (Mackenzie Roberts, undergraduate student researcher working with Dr. Kutzler). There was a weak negative correlation between chute score and average daily gain using a simple linear regression (see figure). The preliminary results from this research were presented as a poster by Mackenzie Roberts in the College of Agricultural Sciences at the Oregon State University College of Agricultural Sciences Experiential Expo in October 2018 (see poster).



GENOMIC TESTING FOR PRODUCTION AND PERFORMANCE TRAITS IN CROSSBRED ANGUS CATTLE

Mackenzie Roberts, Michelle Kutzler

Department of Animal and Rangeland Sciences

INTRODUCTION

- Selection of quality replacement heifers is challenging in commercial cow-calf operations.
- Producers must decide a heifers production value before she is able to express profitable production traits.
- DNA testing may provide useful information regarding a producers ability to make decisions regarding replacement heifers.¹
- The traits of interest include both performance (average daily gain, docility, ribeye area, fat thickness, marbling) and production (birth weight, calving ease, reproductive tract score, number of antral follicles, conception rate, rebreeding rate).
- Commercial cattle genetic testing companies claim accumulative value returns for genetic beef selection of replacement heifers.
- However, there are no current peer-reviewed articles of these claims validating the accuracy of the results of genomic testing expressed by the individual animal.

HYPOTHESES & OBJECTIVES

- The null hypothesis is that DNA testing will provide useful information for making decisions regarding replacement heifers. The alternative hypothesis is that DNA testing will not provide useful information for making decisions regarding replacement heifers.
- The objectives of this study are :
 - To evaluate performance and production traits on crossbred beef cattle
 - To compare these traits to the results of the genomic testing.
- We intend this research will provide unbiased and tested information to support the decision-making process regarding the cost-benefits of genomic testing and how it could affect the cow-calf producer.

COMPLETED METHODS

- Crossbred Angus beef calves (n=94) from the Oregon State Universities Soap Creek Ranch were used for this study.
- The birth weight, birth date, weaning weight, and weaning date were all provided by the ranch manager.
- One day after weaning, a blood sample was collected from the tail vein into EDTA blood tubes (Figure 1). Blood was transferred to blood sampling cards provided by the cattle genetic testing companies (NeoGen and Zoetis), allowed to dry overnight, and then shipped by U.S. mail.
- Two days after weaning, hair over the right 12th and 13th ribs near the dorsal midline and between the hooks and pin over the sacrum was clipped. Alcohol (75%) and ultrasound coupling gel were applied to the skin and three ultrasound images were captured for the purposes of measuring percent intramuscular fat, rib-eye area, back fat thickness, and rump fat thickness (Figure 2).
- During both days, exit velocity score (scale of 1 – 6) was determined as the calves left the chute.²

METHODS IN PROGRESS

- October-November 2018: Performance traits will be calculated from the ultrasound images
- November 2018-January 2019: Genomic testing results will be evaluated
- January – May 2019: Production traits will be determined from 25 heifers selected from this group as replacement heifers



Figure 1. Sampling method for blood collection.

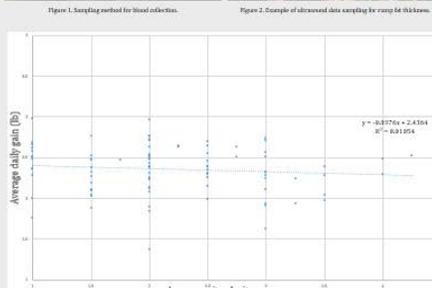


Figure 2. Example of ultrasound data capturing the rump fat thickness.

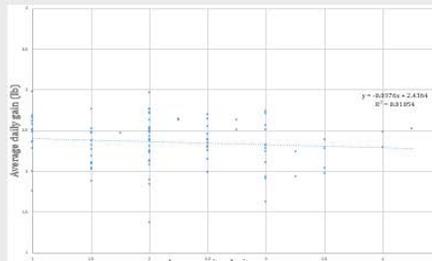


Figure 3. Average daily gain is negatively correlated with average exit velocity scores.

PRELIMINARY RESULTS

- Average daily gain was negatively correlated with average exit velocity score (Figure 3).
- However, this was a weak correlation ($R^2=0.01054$).

TAKE HOME MESSAGE

- We intend this research will provide unbiased and tested information to support the decision-making process regarding the cost-benefits of genomic testing and how it could affect the cow-calf producer.

REFERENCES

1. Snelling WM, Cushman RA, Fortes MR, et al. Physiology and Endocrinology Symposium: how single nucleotide polymorphism chips will advance our knowledge of factors controlling puberty and aid in selecting replacement beef females. *J Anim Sci* 2012;90:1152-1165
2. Beef Improvement Federation Guidelines. Chute scoring guidelines, p. 32 – 33 https://beefimprovement.org/wp-content/uploads/2018/03/BIFGuidelinesFinal_updated0318.pdf

ACKNOWLEDGEMENTS

- Our thanks to the Oregon Beef Council for their generous grant support for this project.
- Special thanks to the Beginning Researcher Program at Oregon State University for giving the opportunity to undergraduate students to learn about ongoing research on our campus, and how it affects our world.



Live carcass traits were evaluated on all 94 calves on the second day after weaning (October 5, 2018) by Dr. Kutzler using real-time ultrasound with a linear-array transducer as described in the Beef Improvement Federation Guidelines. These traits include 12th -13th rib fat thickness, rump fat thickness, ribeye area, and intramuscular fat percentage (marbling). Each of these traits is significant in the determination of quality and red meat yield for individual animals, and each is moderately heritable. Calves were restrained in a squeeze chute with fold-down side panels and had the hair clipped over the scanning area to improve contact between the ultrasound transducer and the area to be examined. Rib fat thickness and rib eye area were measured at the 12th -13th rib at a point ¾ of the distance from the medial end of the longissimus dorsi muscle (12th -13th rib interface). Rump fat thickness is a fat depot that is highly related to 12th -13th rib fat thickness (heritability > 0.70). This measurement can be beneficial when scanning very lean animals (e.g. weanlings) and can be used to improve the overall accuracy of external fat estimation. To collect this image, the ultrasound transducer was placed horizontally between the hooks and pins. Rib and rump fat thickness values were measured with internal calipers within the ultrasound unit. Rib eye area values were measured using an internal tracing function and area calculation within the ultrasound unit. Percent intramuscular fat (% IMF) is a trait that is highly correlated with USDA marbling score. The % IMF measurement was made from an image collected across the 11th -13th ribs (or 12th -13th ribs) at a lateral position from

the animal's midline at a point $\frac{3}{4}$ of the distance from the medial end of the longissimus dorsi muscle. Data from the live carcass evaluation is currently being analyzed.

Whole blood samples were collected from the tail vein of each calf one day after weaning into a vacutainer containing an anticoagulant (EDTA). DNA blood cards were prepared from each blood sample and then samples were shipped to Neogen and Zoetis, where total DNA will be extracted and genotyping will be performed. The estimated processing time for the genomic testing is 4 weeks (results should be available in early November 2018). After the results are available, the Map Viewer tool of the bovine genome (http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9913&build=6) will be used by the Oregon State University Center for Genomic Research and Biocomputing to compare genomic output data with measured performance and production traits. Minor allele frequency (MAF) will be determined using the FREQ procedure of SAS software. Distributions of genotypes will be tested for deviation from Hardy-Weinberg equilibrium using a chi-square test. In addition, chi square will be used to determine whether MAF differed between calves with superior performance traits. The association of genetic variants with each trait will be evaluated using the MIXED procedure of SAS. To determine SNP effects, the genotype will be considered a continuous variable to determine the allele substitution effect. Significance will be defined as $P < 0.05$. All of the animal procedures used in this research were approved by the Oregon State University Animal Care and Use Committee (ACUP #5079).

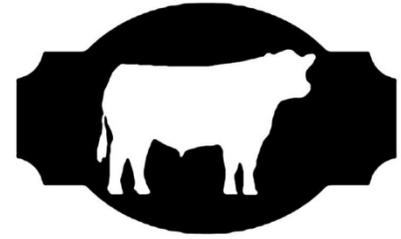
Work Planned for Years 2 and 3:

In Year 2, heifers (n=24) will be evaluated monthly from 12 to 14 months of age by ultrasonography to determine pubertal status. Briefly, all heifers will have their reproductive tract examined transrectally by ultrasonographic evaluation to determine the reproductive tract score (RTS). During the ultrasonographic examination, antral follicles (follicles that are >3 mm) will also be counted as high numbers (>25) of antral follicles are highly correlated with fewer services per conception. At the first of May, all yearling heifers will be treated with CIDRs to allow synchronized artificial insemination (AI). One day following the AI breeding, heifers will be assigned to single-sire natural service cleanup pastures. Pregnancy examination via transrectal ultrasonography will be performed at 60 d after AI breeding. Using the genomic data previously obtained from these heifers at weaning, the Oregon State University Center for Genomic Research and Biocomputing will compare genomic output data with measured fertility traits (e.g. age of puberty, RTS prior to breeding, number of antral follicles, first cycle conception rate). In Year 3 of the proposed research, pregnant heifers will be followed through calving so that birth weight and calving ease can be determined as well as heifer rebreeding. The success rate of rebreeding after first calving is a major issue in beef cattle farming, with reductions of up to 20% in

calving rates reported from the first to the second year in production. Improving the rebreeding rate of heifers should increase the economic efficiency of beef cattle production. These three additional fertility traits will be compared in a similar manner to the genomics data previously obtained from the heifers at weaning. The remainder of Year 3 will be used for final data analysis and manuscript preparation.



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 feeding essential fatty acids 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: August 2019

Project status: One hundred and four Angus-Hereford cows were ranked by body weight, body condition score, and allocated to one of two treatments at 195 days of gestation:

- 1) Control: 200g/cow/day of a rumen protected source of non-essential fatty acids (EnergyBooster, Milk Specialties, Eden Prairie, MN)
- 2) Essentiom: 200g/cow/day of a rumen protected source of essential fatty acids (Essentiom Church and Dwight Co., Inc., Princeton, NJ)

Cows were maintained in two adjacent pastured from day 0 (start of treatments administration) until calving, and each pasture contained the same number of animals from each treatment group (per pasture: Control, n = 26 and Essentiom, n = 26).

Sampling:

Immediately after calving, dam and calf were brought to a working facility where body weight (**BW**) and blood samples were collected from the pair. From the dam, samples of the colostrum and placenta were also collected (when feasible; colostrum n = 103; placenta, n = 38) and body condition score (**BCS**) was assessed. From the calf, a muscle biopsy was collected at time of birth and an additional blood sample was collected 24h after birth to assess antibody absorption from colostrum ingestion. Treatment administration was ceased after calving and cows and calves were moved to a different pasture and managed as a single group. Calves were branded and at approximately 30-45 days of age. At weaning, when calves were on average 208 days of age, calf BW was assessed in two consecutive days, dam BW was also recorded and dam BCS assessed by two trained technicians. For calf body

weight the average between the two days was utilized and for dam BCS, the average between two evaluators.

Partial Results

Calves were weaned in October (2018) and shipped to a commercial feedyard for growing and finishing (Lighting Feeders, Nyssa, OR). Results from calving and weaning are reported in Tables 1 and 2. Project will be completed when calves are slaughtered in (August 2019), and a full report provided including all performance, carcass characteristics, and analyses of biological samples collected during the experiment.

Table 1. Calving results (February to March 2018).

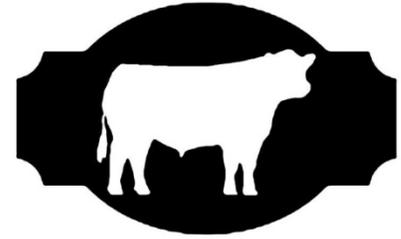
Item	Control	Essentiom	SEM	<i>P-value</i>
Dam BW, kg	1197	1220	21	0.45
Dam BCS	4.7	4.9	0.1	0.08
Gestation length, d	277.3	277.0	0.6	0.71
Calf BW, kg	81.3	82.0	1.2	0.71

Table 2. Weaning results (October 2018).

Item	Control	Essentiom	SEM	<i>P-value</i>
Dam BW, kg	1250	1239	20	0.71
Dam BCS	5.2	5.1	0.08	0.43
Calf age, d	208.8	208.7	0.07	0.66
Calf BW, kg	575	581	9	0.68



Oregon Beef Council Report



Beef Cattle Sciences

Livestock riparian guidelines may not promote woody species recovery where wild ungulate populations are high ¹

Bryan A. Endress², J. P. Averett³, and M.J. Wisdom⁴

Synopsis

Stubble height, streambank alteration, and woody species use are indicators used to monitor livestock impacts on riparian areas in the western United States. Effects of wild ungulates on riparian conditions are often not monitored and assumed to be represented by indicators developed for livestock. We tested this assumption by evaluating effects of elk (*Cervus canadensis*) and mule deer (*Odocoileus hemionus*) on grazing indicators along Meadow Creek, a salmonid-bearing stream in northeastern Oregon. Wild ungulates reduced stubble height by 20-30%. Mean streambank alteration was 1.1% (ranged from 0.3-8%). Woody species use was negatively related to stubble height and positively related to streambank alteration ($p < 0.05$). Despite maintenance of stubble height and streambank alteration within regulatory guidelines, wild ungulate use of preferred woody species was moderate to high (> 50%). Adherence to guidelines developed for livestock may not result in desired riparian conditions where wild ungulate populations are high.

Summary

The objective of this experiment was to evaluate the effects of acclimation to human handling on growth, plasma concentrations of cortisol, and puberty attainment of Brahman-crossbred heifers.

Over two consecutive yr, 37 Braford and 43 Brahman × Angus heifers were assigned randomly to receive or not the acclimation treatment within 30 d after weaning. The acclimation process consisted of bringing heifers to the cowpens three times weekly during four consecutive wk, where heifers were exposed to common handling practices and returned to pastures within 2 h. Heifers were maintained in bahiagrass (*Paspalum notatum*) pastures and received a blend of soybean hulls and cottonseed meal at a daily rate of 6.0 lbs of DM per heifer during the experiment (d 0 to 130). Blood samples were collected prior to and at the end of the acclimation process for determination of cortisol concentrations. Puberty status was assessed monthly during the experiment. Acclimated heifers had decreased ($P < 0.05$) average daily gain (ADG) compared to control heifers (1.1 vs. 1.3 lbs/d, respectively). Attainment of puberty, however, was hastened ($P < 0.01$) for acclimated heifers. Further, acclimated heifers had reduced cortisol concentrations compared to control heifers after the acclimation period (3.8 vs. 5.1 $\mu\text{g/dL}$, respectively). Results from this experiment indicated that although acclimation decreased body weight gain, it enhanced the attainment of puberty in Brahman-crossbred heifers.

Introduction

Active monitoring is necessary to prevent negative impacts of ungulates on riparian areas.

Because domestic (Kauffman and Krueger 1984) and wild ungulates (Danell 2006) can have profound impacts on riparian vegetation composition and structure, monitoring indicators must reflect the effects of all ungulates—domestic and wild—on riparian systems. However, wild ungulate effects are often not monitored, ignored, or assumed to be represented by indicators developed for livestock.

Stubble height, streambank alteration (alteration), and woody species use are the most common indicators used to manage livestock grazing in riparian areas. The simplicity and repeatability of these measurements has prompted their use as catch-all guidelines to indicate grazing effects and overall riparian health for some land management and regulatory agencies (University of Idaho Stubble Height Review Team 2004; Roper 2016). Stubble height can be a good indicator of livestock effects on woody species, sediment trapping, and short-term recovery potential of grass/grass-like species in some situations (Skinner 1998; Clary Leininger 2000). At landscape scales, stubble height and alteration can be useful indicators of cattle effects in riparian areas and correlate with stream conditions important for salmonids (e.g., bank angle, percent pools, pool depth; Goss and Roper 2018). However, stubble height and alteration relationships with wild ungulate impacts on riparian vegetation have not been studied. Because woody shrubs compose a larger percentage of elk and deer diets compared to cattle (Hofmann 1989), reliance on indicator guidelines developed for cattle (e.g., minimum stubble height) may not protect woody species from over-browsing by wild ungulates. Our objectives were to evaluate the effects of elk and deer herbivory on riparian stubble height, and to examine the relationship between both stubble height and alteration with woody species use by elk and deer along a cold-water, salmonid stream.

Materials and Methods

This study took place along Meadow Creek within the Starkey Experimental Forest and Range (SEFR) in northeastern Oregon (45° 12' N, 118° 3' W). SEFR elevations range between 1120-1500m, annual average temperatures range from -4°C (winter) to 18°C (summer), and average annual precipitation is 510mm (Rowland et al. 1997). SEFR vegetation includes shallow-soil bunchgrass communities, and *Pinus ponderosa*/*Pseudotsuga menziesii*, and *Abies grandis*/*Pseudotsuga menziesii* forests. Dominant herbaceous species along Meadow Creek included *Agrostis stolonifera*, *Scirpus microcarpus*, *Carex*

pellita, and the most abundant woody species were *Alnus incana* and *Crataegus douglasii*.

The Meadow Creek study area served as spring-fall range for elk (density = 5.6-6.8 per km²) and mule deer (2.8-3.6 per km²; Ager et al. 2003). Population estimates equate to approximately one elk per 36-44 acres and one deer per 68-88 acres. Additionally, elk use of the Meadow Creek riparian area—around the time of this study—was estimated to be ~ 11 times greater than that of mule deer (Averett et al. 2017). Wild ungulate use along Meadow Creek was sparse in winter because most animals migrated in late fall-early winter to the SEFR winter area (Rowland et al. 1997). Livestock grazing did not occur during the period of our study.

We sampled vegetation along the “greenline” within 16 designated monitoring areas (DMAs; each ~ 150 m in length) using the Multiple Indicator Monitoring Protocol (Burton et al. 2011). Twelve DMAs were located within three different stream reaches that coincided with herbivory treatments - six excluded from wild ungulates using 2.4 m tall fencing, and six exposed to wild ungulates. Four additional DMAs (exposed to wild ungulates) were established to increase coverage along the entire 11 km reach. Stubble heights of graminoids (grasses, sedges, and rushes), alteration, and woody species use were measured simultaneously within quadrats composed of two adjoining plots (50 X 20 cm) placed at 2.5m increments along the greenline on both sides of the stream (n ~ 120 plots per DMA).

Stubble height was measured for each species nearest the frame handle to minimize bias using the method in Burton et al. (2011). Alteration was measured using a 5-line intercept method described by Burton et al. (2011), and calculated as the percentage of plot lines that intercepted depressions/hoof prints/trails. Woody species use was measured as percentage of current year’s leaders browsed (Burton et al. 2011) for species rooted within 1 m of the green-line, classified into categories: one (0-20%); two (21-40%); three (41-60%); four (61-80%); and five (81-100%), and limited to plants available for browsing (> 50% of photosynthetic area below 2.5 m; Burton et al. 2011). Sampling occurred in mid-summer (late-July 2015) to coincide with peak production when species were easiest to identify, and then repeated in late-October 2015 at the end of the growing season.

We compared mean and median stubble heights (July and October separately) across herbivory treatments using randomization tests. A randomization test calculates the proportion of test

statistics (difference in mean/medians between groups) determined from a large number of random permutations (10,000), into groups of the same size as observed, that are \geq to the observed test statistic (Manly 1991). We limited comparisons to treatment areas ($n = 12$; six protected and six unprotected) because the other four DMAs were spatially distant from the protected DMAs, and may not be comparable. Comparisons were made for all graminoids and then again for dominant species (five most abundant species based on plot frequency). Median stubble height was evaluated because it is less sensitive than the mean to a few extreme values. To compare herbivory effects by species, percent change (unprotected – protected) in stubble height was calculated for the ten most abundant graminoids.

Mean deciduous woody species use (arithmetic midpoint of use class) was calculated for all species combined and then separately for species preferred by elk and deer, i.e. *Cornus sericea* (dogwood), *Populus balsamifera* (cottonwood), and *Salix* (willow) species. Chronic herbivory can result in species composition shifts where sensitive and/or preferred woody species decline, and tolerant and/or less preferred species become dominant (Rooney and Waller 2003). Therefore, use of less preferred species, e.g., *Alnus* spp. and *Crataegus douglasii*, may underestimate herbivore impacts (Rooney and Waller 2003). Use of preferred species was of special interest because cottonwoods and willows are functionally important to western riparian areas (Patten 1998), making them key restoration species along salmonid streams. One DMA did not contain preferred species, resulting in a DMA sample size of 15 for preferred species. Boxplots of woody species use, stubble height, and alteration were interpreted in relation to common regulatory guidelines for grazing in the region (USDA Forest Service 2016; National Marine Fisheries Service 2017). Linear regression was used to explore relationships between woody species use and stubble height and alteration. Models were fit for all and preferred species separately. The alteration data had one extreme outlier (8.26%; 3.5 Standard deviations above the mean of 1.1%) that was excluded from this analysis.

Results

Mean stubble height was greater ($p < 0.001$) in protected sites compared to unprotected sites (Fig. 1). The treatment effect (mean protected stubble height – mean unprotected stubble height) was small in July (all species = 2.1 cm, 95% CI = 0.8-3.6 cm;

dominant species = 2.8 cm, 95% CI = 1.2-4.5 cm), but increased substantially by October (all = 8.0 cm, 95% CI = 6.3-9.8 cm; dominant species = 10.9 cm, 95% CI = 8.8-13.1 cm; Fig. 1). Median stubble height did not differ between treatments in July, but was higher ($p < 0.001$) for protected sites in October (all = 9.6 cm, 95% CI = 8-11 cm; dominant species = 12.9 cm, 95% CI = 11-15 cm; Fig. 1). Wild ungulates reduced end of growing season greenline stubble heights by ~8-10 cm (~ 3-4 inches) for all species and by ~11-13 cm (~ 4-5 inches) for dominant species, corresponding to height reductions of 20-30% (Fig 1). October stubble heights in DMAs unprotected from wild ungulates varied from 22-40 cm (Fig 1). Variation was likely due to site factors, plant species composition, and wild ungulate disturbance. Wild ungulate effects on stubble height may have different implications for livestock management depending on site conditions. For example, in stream sections with inherently shorter stubble heights, wild ungulate disturbance may pose challenges for compliance with grazing guidelines (National Marine Fisheries Service 2017). Research is needed to determine if the introduction of livestock into our riparian system will add to wild ungulate effects, or if wild ungulates will shift distributions in response to livestock presence (Coe et al. 2001).

Wild ungulates reduced the height of the ten most abundant graminoids (Table 1). Percent height change ranged from -23.7% (*Agrostis stolonifera*) to -3.5% (*Eleocharis palustris*; Table 1). Variation by species was likely due to a combination of herbivore preference, species composition, and differential regrowth (following herbivory) among species. Monitoring protocols often focus on measuring stubble height for “key species”, e.g., important forage, or species sensitive to grazing (Burton et al. 2011). We found that stubble heights were highly variable along Meadow Creek, depending on the species selected for monitoring, reinforcing the concern that monitoring just a few key species may not represent overall vegetation trends (Roper 2016).

Woody species use in unprotected DMAs ranged from 19-69% for all species (mean = 34.6%; SD = 17.3%), and from 34-90% for preferred species (mean = 57.8%; SD = 16.6%; Figs. 1 & 2). Use was moderate to heavy (>40% of leaders browsed) in three out of ten (30%) DMAs for all species, and in eight out of nine (89%; Fig. 2) for preferred species despite maintenance of: (1) streambank alteration well below (mean = 1.1%; SD = 2.0%) the regulatory guideline (< 20%; Fig 1); and (2) stubble

heights that were above the minimum 15 cm guideline (USDA Forest Service 2016; National Marine Fisheries Service 2017; Fig 1). Previous research at Meadow Creek provides evidence that the current browsing pressure by wild ungulates is suppressing the establishment of woody species, particularly preferred species, e.g., willow and cottonwood (Case and Kauffman 1997; Averett et al. 2017). This is of particular concern because cottonwoods and willows are among the few riparian species able to attain heights tall enough to provide substantial stream shading. Consequently, cottonwood and willow species are important for moderating high late-summer stream temperatures, a major limiting factor for salmonid populations in systems like Meadow Creek (McCullough 1999). Other studies have also demonstrated suppression or alteration of riparian woody communities by wild ungulates in the absence of livestock (Kay 1994; Danell 2006). Our results suggest that, even if riparian monitoring takes place, compliance with grazing guidelines may not protect preferred woody species from high browsing pressure by wild ungulates.

Woody species use was negatively related ($p < 0.01$) to stubble height and positively associated ($p < 0.01$) with alteration (Fig. 2); there was slightly less evidence ($p = 0.07$) for a relationship between preferred woody species use and alteration. Results suggest that estimates of stubble height and alteration may provide a coarse indication of woody species use. Our ability to detect relationships between use and stubble height/streambank alteration was limited by a small sample size ($n=16$ DMAs). Future analyses with greater sample size, increased spatial scale, and consideration for wild ungulate distributions and woody species composition will increase our understanding of these relationships. Nonetheless, our results indicate that maintenance of stubble heights above 35-40 cm (~14-16 inches) and streambank alteration below even 5% may not protect preferred woody species from moderate to heavy browsing ($> 40\%$ leaders browsed) by wild ungulates in this system.

Conclusions

Wild ungulates contributed measurable reductions to greenline stubble height. Managers should consider monitoring wild ungulate impacts on herbaceous species in riparian areas to inform decisions related to grazing implementation and/or duration. As our results demonstrate, adherence to

regulatory guidelines designed for managing livestock in riparian areas may not protect functionally important woody species, i.e., willows, cottonwoods, from over-browsing in similar systems with high populations of wild ungulates.

Acknowledgements

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Table 1: Stubble height summary statistics for all, dominant, and the ten most abundant graminoid species, ordered top to bottom by rank abundance.

Species	Mean (cm)		Change (cm)	%Change	Sample size		STDEV (cm)	
	Protected	Unprotected			Protected	Unprotected	Protected	Unprotected
All	44.2	36.2	-8.0	-18.1	1612	1178	22.2	21.8
Dominant	43.2	32.3	-10.9	-25.2	909	765	20.9	19.4
<i>Agrostis stolonifera</i>	30.0	22.9	-7.1	-23.7	336	366	17	14.3
<i>Scirpus microcarpus</i>	47.2	36.5	-10.7	-22.7	217	149	14.9	14.8
<i>Carex pellita</i>	48.9	40.8	-8.1	-16.6	184	108	12.8	15.8
<i>Elocharis palustris</i>	31.7	30.6	-1.1	-3.5	173	84	9.8	10.1
<i>Carex lenticularis</i>	40.7	33.9	-6.8	-16.7	84	82	13.5	15.3
<i>Juncus ensifolius</i>	29.6	28.0	-1.6	-5.4	105	47	9.8	11.3
<i>Alopecurus pratensis</i>	74.5	60.9	-13.6	-18.3	88	60	24.1	26.5
<i>Glyceria striata</i>	61.2	55.8	-5.4	-8.8	61	61	23.5	29.1
<i>Phleum pretense</i>	56.7	52.6	-4.1	-7.2	65	55	26.4	27.7
<i>Juncus arcticus</i>	60.0	57.5	-2.5	-4.2	64	30	14.1	15.0

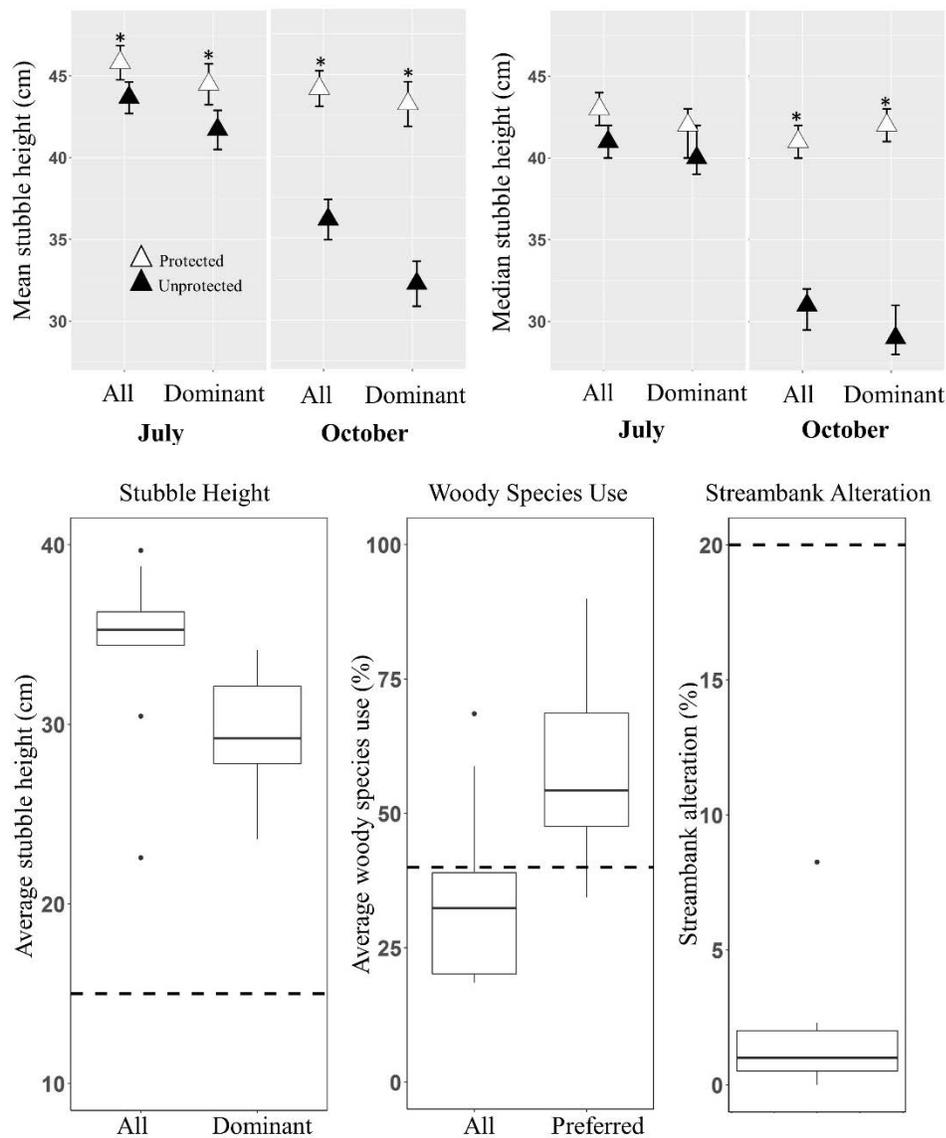


Figure 1: (Top panel) Stubble heights (protected/unprotected from wild ungulates) for all and dominant graminoid species. Error bars show 95% confidence intervals. Asterisks indicate significance ($p < 0.05$). (Bottom panel) End of growing season stubble height, woody species use (all and preferred species), and streambank alteration for unprotected DMAs. Boxes show 1st and 3rd quartiles. Whiskers extend 1.5 times the interquartile range; points show data beyond the whiskers. Dashed lines: (left panel) minimum stubble height guideline, 15 cm; (middle panel) woody species use guideline, $< 40\%$; (right panel) maximum alteration guideline, 20% .

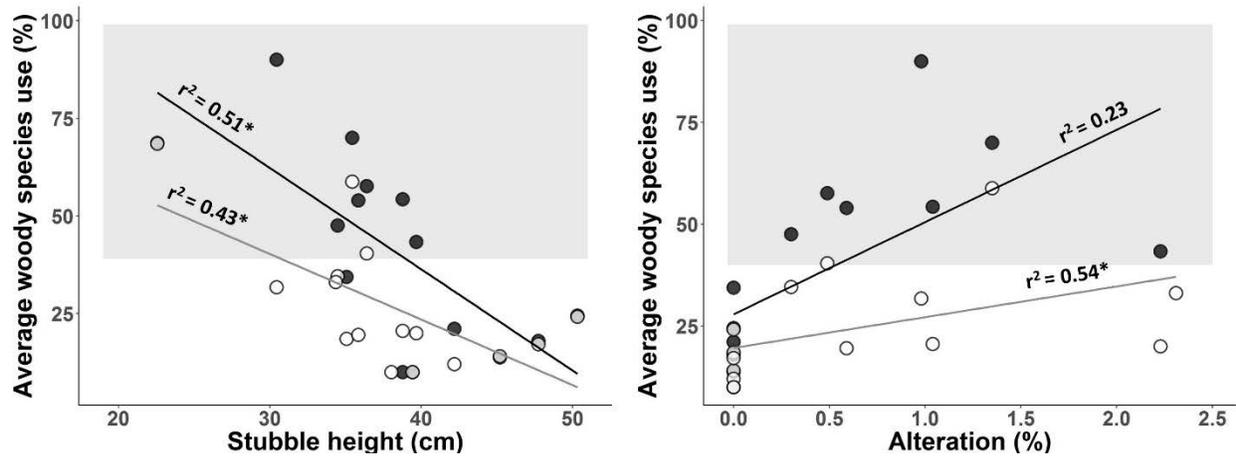
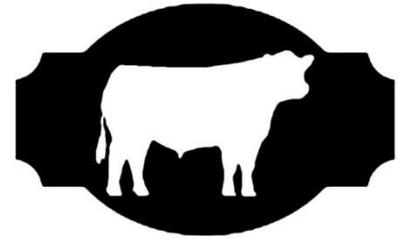


Figure 2. Average woody species use against stubble height and streambank alteration (Black, preferred species; white, all species). Linear regression lines (black, preferred species; grey, all species; shaded area, use above regulatory guideline (> 40%). Asterisks indicate significance ($p < 0.05$).



Oregon Beef Council Report



Beef Cattle Sciences

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

Carlos G Ochoa²

Synopsis

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

Summary

The present report shows results from ongoing field data collection and analyses of multiple vegetation and hydrology-related variables. Overall, greater herbage response was observed in the treated watershed when compared to the untreated. Juniper saplings in the treated watershed ranged from 1 to 15 years old, averaging 9 years. In the untreated watershed, we found that canopy cover is nearly 30% and 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 ranged 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 [1,2] and grassland ecosystems[3,4] 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 [5–7], reduce biodiversity [8–10], alter soil nutrient cycling [11], and modify hydrologic processes such as evapotranspiration and soil moisture [12–14]. 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.

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 documented juniper canopy cover and density in both the treated watershed and the untreated watershed. We used the belt transect method to determine juniper sapling stem density and canopy cover in the treated watershed. Also, we used a combination of low-altitude imagery, satellite imagery, and on-the-ground measurements (i.e., line intercept and densiometer readings) to document tree canopy cover in both watersheds. Various juniper saplings were removed to extract cross-sections used to determine tree age using ring count techniques. We estimated species composition and herbage production at the end of the growing season in 2018 in both watersheds (Figure 2). In addition, we analyzed the relationships between surface water and groundwater sources in both watersheds and in the riparian valley using graphic and statistical analyses.

Results

Study results indicate there is a density of 126 saplings per acre in the treated watershed, with an average sapling age of 9 years, ranging from 1 to 15 years old. Total juniper density including mature trees and saplings in the untreated watershed is 322 trees/acre, this is similar to what it was previously reported in 2004, before the removal of juniper in 2005. Total tree canopy cover occupancy is <1% in the treated watershed and near 30% in the untreated watershed. Herbage production was estimated at

560 lb/acre in the treated watershed and at 386 lb/acre in the untreated.

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. Based on-the-ground measurements, canopy cover was determined to be 30% in the downstream location and 28% in the upstream. We also tested different techniques using low altitude imagery collected by the UAV systems and results showed 33% canopy cover for the downstream location and 27% for the upstream location. When using satellite imagery, canopy cover was 29% at the downstream location and 26% at the upstream (Figure 3).

Juniper-canopy cover interception at the untreated watershed accounted for 46% (downstream plot) and 36% (upstream plot) of total annual precipitation.

We continue to test the use of multispectral imagery obtained from low altitude flights from UAV systems. We used low altitude aerial imagery in combination with on-the-ground measurements to provide a better visual representation of juniper reestablishment throughout the landscape (Figure 4).

Our ongoing findings show that vegetation indices calculated from multispectral imagery using UAVs flying at 60 m can help identify juniper saplings from other types of vegetation. This was more evident during the fall season, when most other vegetation is senescing. Results of this research, and past studies, indicate juniper encroachment can result in shifts in vegetation density and composition. Given the large scale of juniper encroachment, the use of UAVs offers the advantage of more efficient data collection compared to using ground-based techniques alone. In contrast to satellite-based techniques, UAVs offer a high-resolution, flexible platform that can be used to target specific study sites and objectives. Initial results suggest that UAVs can be effectively used to assess vegetation characteristics and tree density in juniper-dominated systems.

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 5

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 5).

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 6). 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).

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

Conclusions

Overall, greater forage response and greater streamflow and springflow levels were observed in the treated watershed. Results from this ongoing study indicate that juniper tree density is greater in the untreated watershed. However, the relatively large number of saplings that had emerged after juniper removal in 2005 is of concern; findings suggest that a secondary treatment is necessary to prevent juniper growth to a mature stage before can decrease site productivity. The use of multispectral imagery using low altitude UAV systems yielded satisfactory results to estimate tree canopy cover and to identify juniper saplings. The combination of on-the-ground techniques and UAV-based information can provide useful information to better estimate juniper reestablishment. The analyses of hydrologic variables indicates there are 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.

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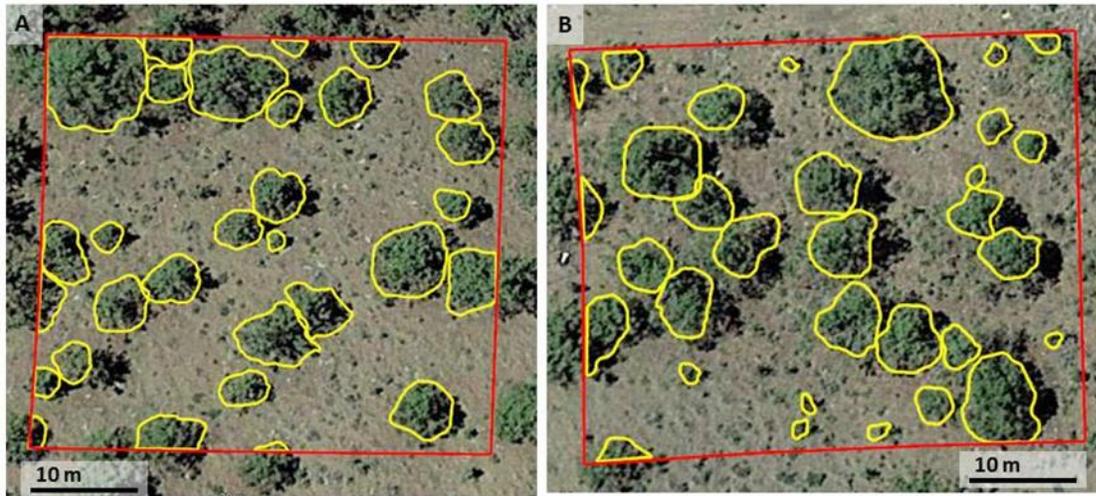


Figure 3. Satellite images illustrating juniper trees (outlined in yellow) used in estimating total canopy cover for the downstream (A) and upstream (B) experimental plots.



Figure 4. Low altitude aerial image illustrating the belt transect method and some of the juniper saplings commonly found in the treated watershed.

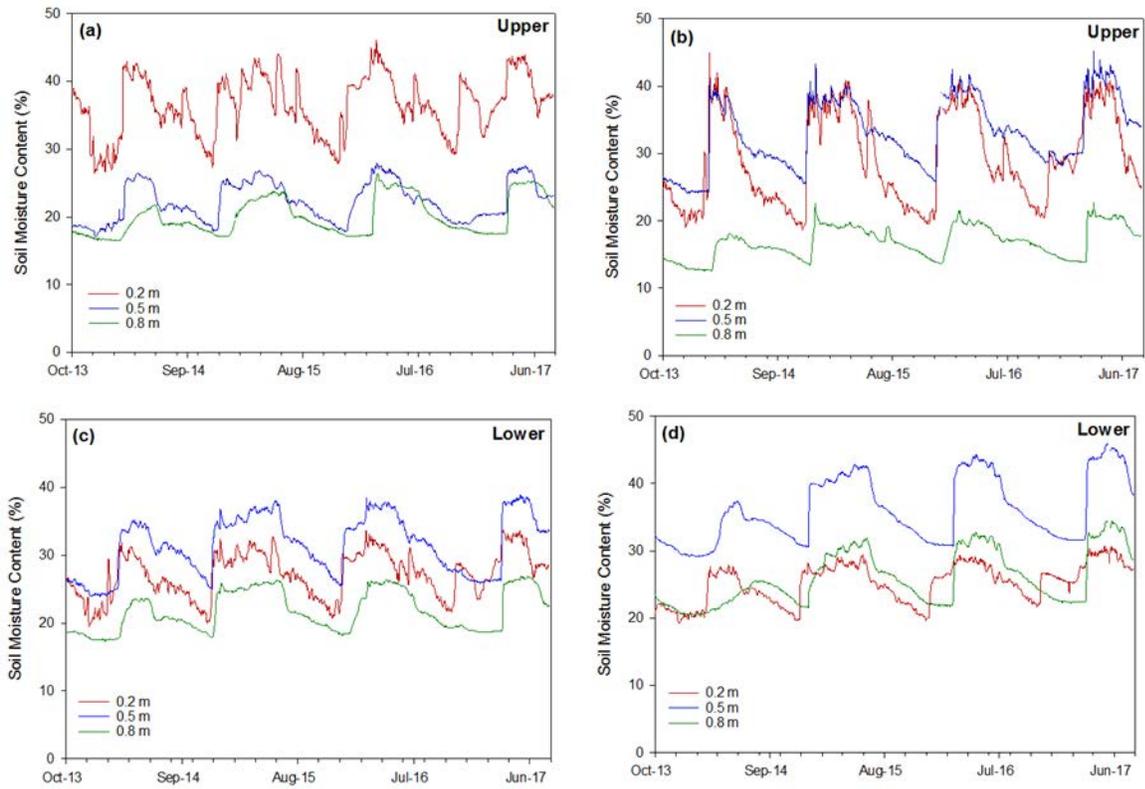


Figure 5. Soil moisture content fluctuations at different soil depths at upper and lower locations in both Mays WS (a, c) and Jensen WS (b, d) watersheds from 1 October 2013 through 27 July 2017.

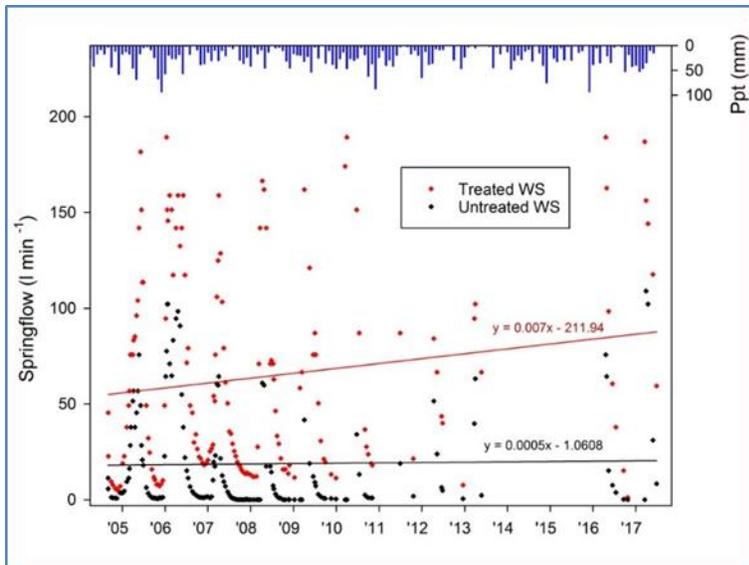
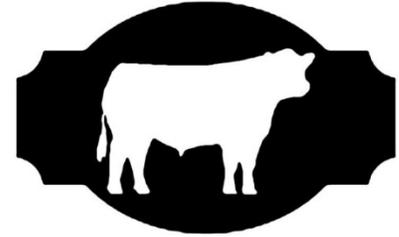


Figure 6. Springflow rate estimates for selected dates and monthly precipitation (Ppt) totals obtained for both watersheds (WS) from September 2004 through June 2017.



Oregon Beef Council Report



Beef Cattle Sciences

How much water do mature and sapling juniper trees really use?¹

Mohamed A.B. Abdallah², Ricardo Mata-Gonzalez², Carlos Ochoa²

Synopsis

Mature juniper trees used about 100 times greater water use than sapling juniper trees. The greatest water demand was in a wet year (2017) compared to a dry year (2018) for both juniper tree types.

Summary

Juniper control is a common practice in many encroached areas of Oregon. However, water use by mature juniper trees and by sapling juniper trees (those resulting after years of juniper control) has not been investigated. Such information is important to assess how much water can be saved by juniper control after years of juniper regrowth. We installed automated water use equipment to monitor whole plant water use in mature trees where juniper has not been controlled as well as in sapling trees in an area where juniper was controlled in 2005 but regrowth has occurred. The water use equipment that we used is based on sap flow measurements by heat transfer and it determines whole-plant water use. The results indicated that mature trees use between 97 and 112 times more water than sapling trees depending on the precipitation (year). The period of highest water use of juniper trees is the summer, followed by the spring and finally the fall. In summary, juniper control results in considerable water savings. Even after a 13-year period of juniper regrowth, the amount of water used by single mature trees can be about 100 times higher than the amount of water used by sapling trees.

Introduction

Woody plant encroachment into semiarid grasslands and savannas is a globally occurring trend over the past 150 years (Andela et al., 2013; Elkington et al., 2014). These structural changes have altered ecosystem processes such as rates of carbon sequestration, soil nutrient cycles (Hibbard et al., 2003) and hydrological processes (Huxman et al., 2005; Ochoa et al., 2018). Woody plants commonly have deeper roots than herbaceous species and as a result are assumed to have greater access to plant available water in water-limited environments (Elkington et al., 2014). Woody plants such as juniper, mesquite and oak can send roots into cracks in the limestone, possibly getting access to water sources that grasses cannot (Elkington et al., 2014). Juniper was found to remain alive without deep taproots, depending instead on an extensive system of shallow fibrous roots where geology prevented deep roots to develop (Thurow and Hester, 1997).

Juniper encroachment in eastern Oregon is reported to increase from 600,000 hectares to over 2.4 million hectares since 1930s. Juniper encroachment into the sagebrush steppe can potentially alter composition, structure, and productivity of understory vegetation (Miller et al., 2005). The understory plants and the forage base for livestock declines at the time juniper starts to control plant community (Miller et al., 2005). Also, soil resources become less available as juniper encroaches (Bates et al., 2005). Moreover, due to the reduction in forage base and the change in natural habitat, rangelands being encroached might be

less capable of supporting native wildlife such as sage grouse (Miller et al., 2005).

One factor related to juniper encroachment, water use by mature and sapling 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 regrowth of sapling 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 sapling juniper trees that result after juniper control, and 3) To assess the potential water savings in areas with juniper control after regrowth with respect to areas with intact mature juniper encroachment.

Materials and Methods

Our research site is known as the paired juniper watershed and it has been studied since 2005. This research site is located in the Camp Creek watershed (lat 43.96N, long 120.34W) in central Oregon. The study site comprises an area of approximately 220 ha and includes two adjacent watersheds, one treated (~ 90% of the juniper removed) and the other untreated (mature juniper remains in the area). In 2005, juniper trees <140 years of age were cut from the treated watershed, and the boles were removed with the remaining limbs scattered. Old growth juniper trees and those that were host to wildlife were not removed. Each watershed is approximately 110 ha with elevations ranging from 1370 m to 1524 m. The wet season in the study area occurs between September and April. Although our study area has been used for monitoring vegetation response and aquifer response to the elimination of juniper, the actual water use by juniper trees has not been evaluated. Furthermore, the water use by sapling juniper trees that result after more than 13 years of treatment has not been evaluated anywhere.

Sap flow measurements were taken for juniper trees in both watersheds. Sap flow was determined in two manners: 1) For large trees (mature) (Fig. 1) using the thermal dissipation probe (TDP) technique (Smith and Allen, 1996; Bladon et al., 2006; Davis et al., 2012) and 2) For small trees (sapling) (Fig. 2) using the stem heat balance (SHB) technique (Baker and Van Bavel, 1987; Smith and Allen, 1996). Mature juniper sapwood area, which is a necessary parameter to calculate whole-tree water use, was estimated from leaf area using a preestablished relationship ($R^2 = 0.99$) (Miller et al., 1987). Measurements for small regrowth trees covered the period from June to November 2017

and then from April to October 2018. Measurements for mature trees covered the period from May to October 2017 and then from April to October 2018. Winter measurements of sap flow are not possible because low temperatures interfere with the correct functioning of the sap flow equipment.

Results

Mature Trees

Water use peaked during the summer months. The maximum water use, around 140 liters (37 gallons) per tree/day was observed in July and August 2017 (Fig. 3). The minimum water use, around 20 liters (5 gallons) per tree/day was observed in October 2018. In both years, the maximum water use was observed during the summer months while the minimum water use was observed towards the fall. This is consistent with Angell and Miller (1994) who reported the greatest juniper transpiration during the summer. In average, water use was higher in 2017 (101 liters per tree/day) than in 2018 (62 liters per tree/day), likely because of the higher precipitation registered in 2017. During the period of highest water uptake, mature trees used 1.6 and 2.9 times more water in July and August 2017 than the corresponding months of 2018.

Sapling (Regrowth) Trees

Similar to mature trees, water use was generally higher during the summer months for juniper saplings (Fig. 4). The maximum water use, around 1.5 liters (0.4 gallons) per tree/day was observed in August 2017. The minimum water use, approx. 0.15 liters (0.04 gallons) per tree/day was observed in November 2017 (there was no measurement for November 2018). Similar to mature trees, water use was lowest during the fall months in saplings. Likewise, as observed in mature trees, water use was higher in 2017 (average 1.05 liters per tree/day) than in 2018 (average 0.55 liters per tree/day) in saplings.

Water Use Comparison

In both tree types, the difference in precipitation year drove large differences in water use. Higher precipitation likely caused larger soil water reserves (Mollnau et al., 2014) that were available for plant uptake in 2017 compared to 2018. However, as expected, the largest difference in water uptake was observed between mature and sapling trees. During the period of highest water use (July) mature trees obtained 90 to 140 times more water than saplings. In average, in a dry year such as 2018 mature trees would use 112 times more water than saplings

whereas in a wet year such as 2017 mature trees would use 97 times more water than saplings.

Conclusions

Here, we document that:

1. Water use by whole juniper trees is driven by the amount of precipitation, which in turn may greatly vary by year.
2. The period of highest water use of juniper trees is the summer, followed by the spring and finally the fall months.
3. Juniper control results in considerable water savings. Even after a 13-year period of juniper regrowth, the amount of water used by single mature trees can be about 100 times higher than the amount of water used by re-growing saplings.

Acknowledgements

This research study was partially funded and supported by the Oregon Beef Council and Oregon State University.

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Figure 1. Installation of automated water use equipment in the field for the area dominated by large trees.



Figure 2. Installation of automated water use equipment in the field for the area where small sapling trees that result after regrowth dominate.

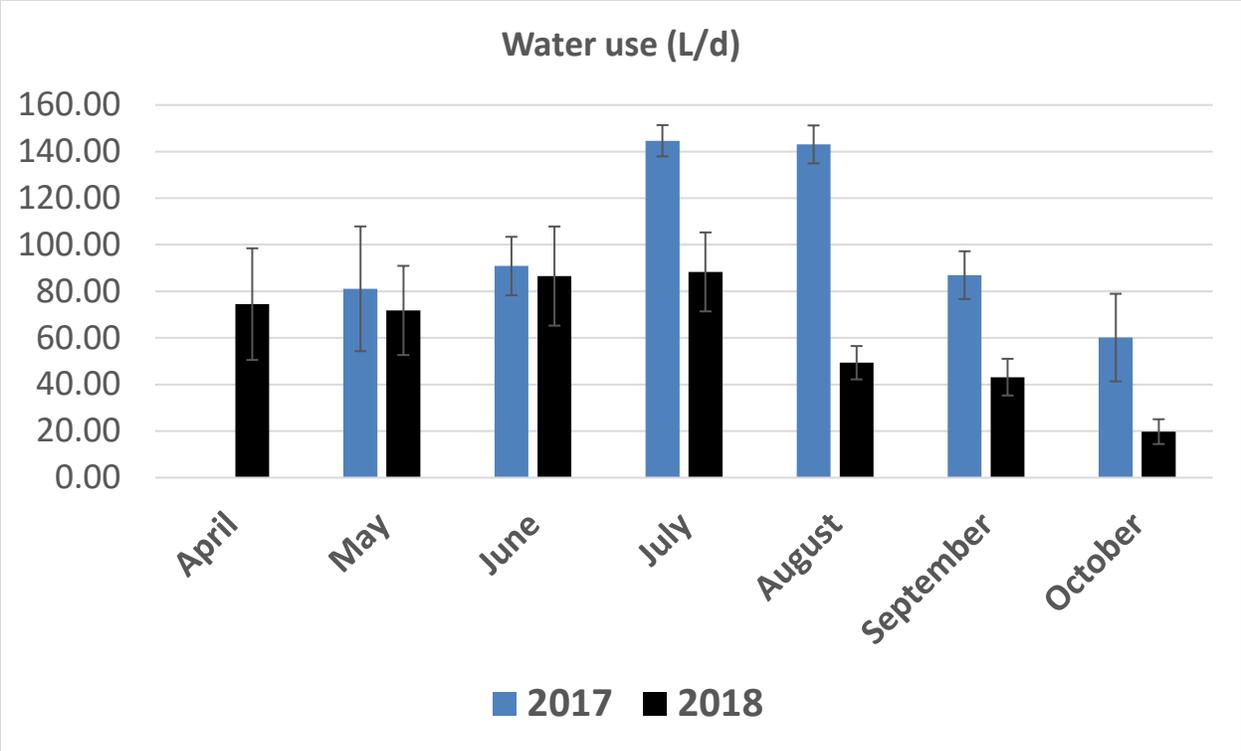


Figure 3. Water use by whole tree in large mature juniper trees in 2017 and 2018.

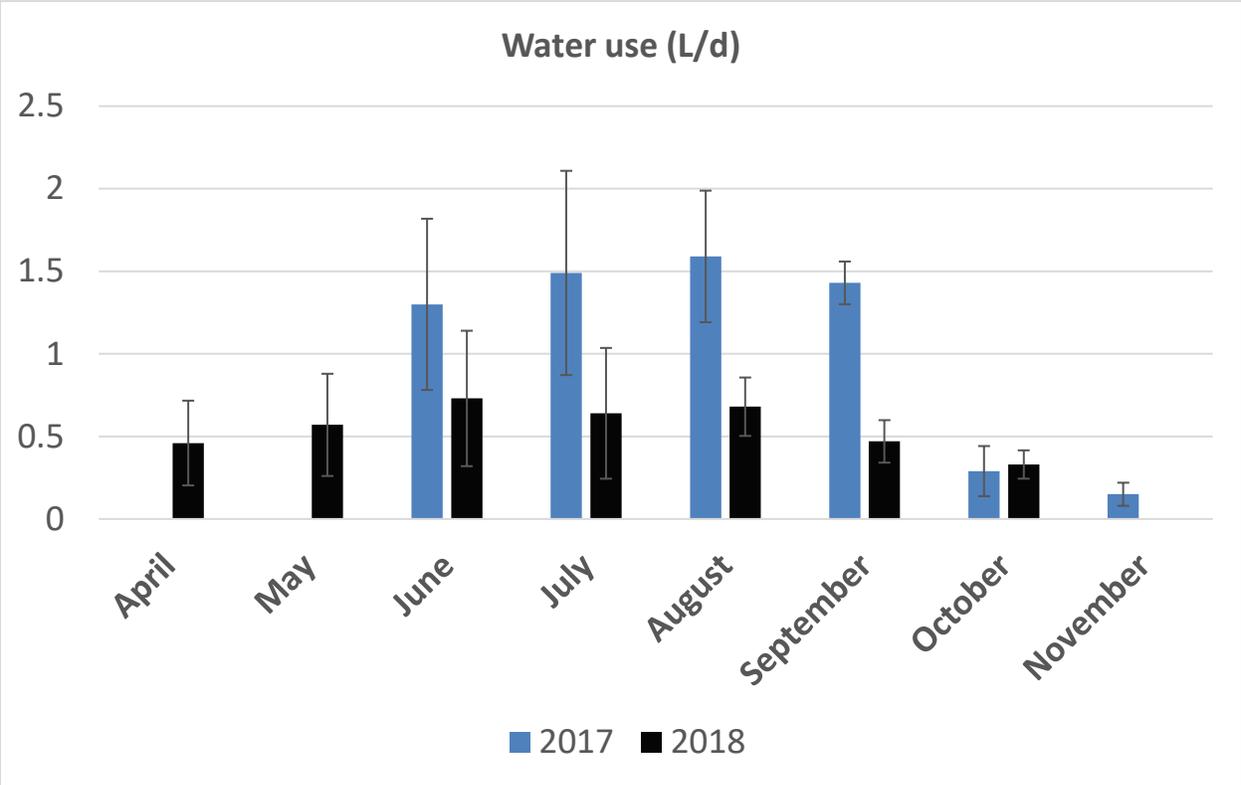
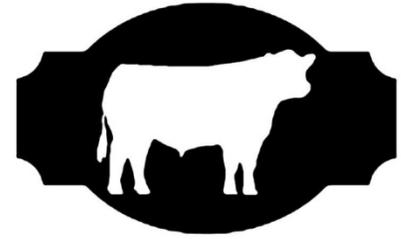


Figure 4. Water use by whole tree in small regrowth juniper trees in 2017 and 2018.



Oregon Beef Council Report

Progress Reports – Rangeland Ecology and Management ¹

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

Contact Person: Sergio A. Arispe, Dustin Johnson, and Kirk Davies

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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 glyphosate (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 (November 2017; OBC & OSU-funded). These treatments were 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 mid-February and early March 2018,

Wyoming big sagebrush was broadcasted on native experimental plots while immigrant forage kochia was broadcasted on introduced seeding treatments (Figure 1). Limited precipitation suppressed plant growth, which delays the nutritional value of clippings until April through November 2019. Figures 2-7 highlight an example of all treatments and seedings at on location.

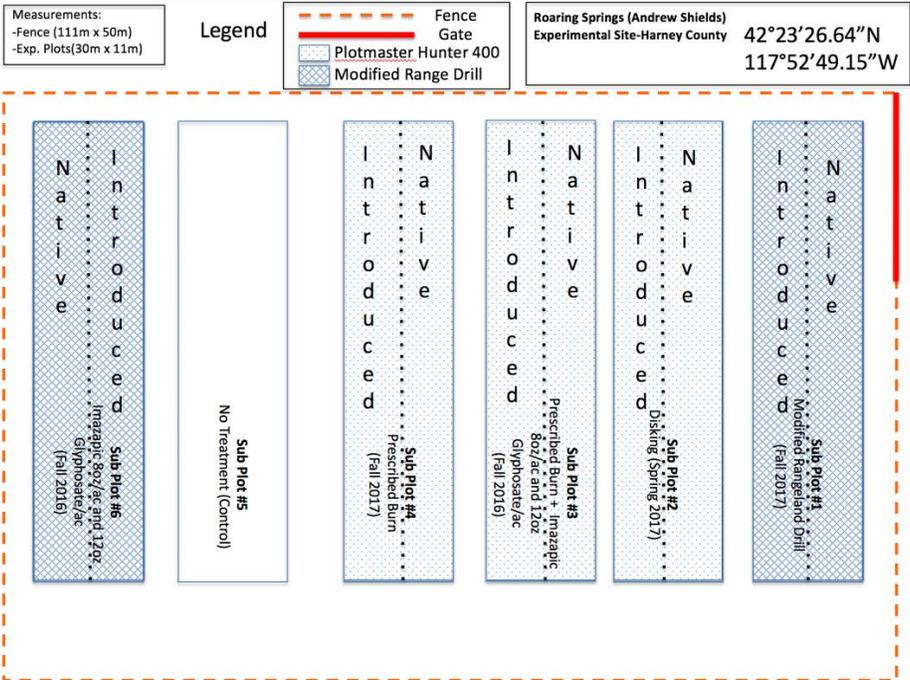


Figure 1: Developing conservation measures fire, mechanical, and chemical treatments and seeding treatments.



Figure 2: Modified rangeland drill treatment with introduced seeding (left) and native seeding (right).



Figure 3: Spring 2017 disking treatment with native seeding (left) and introduced seeding (right).



Figure 4: Fall 2016 prescribed burn with imazapic (8 oz/acre) plus glyphosate (12 oz/acre) with introduced seeding (left) and native seeding (right).



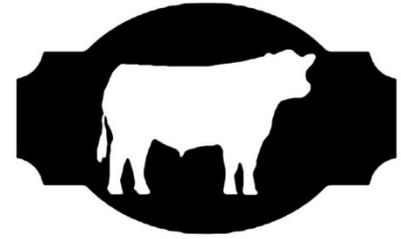
Figure 5: Fall 2017 prescribed burn with native seeding (left) and introduced seeding (right).



Figure 6: Control without disturbance or seeding.



Figure 7: Fall 2016 imazapic (8 oz/acre) plus glyphosate (12 oz/acre) with native seeding (left) & introduced seeding (right).



Oregon Beef Council

Report

Progress Reports – Rangeland Ecology and Management ¹

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

Contact Person: Lesley Morris – OSU Agriculture and Natural Resources Program at Eastern Oregon University

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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 have 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.

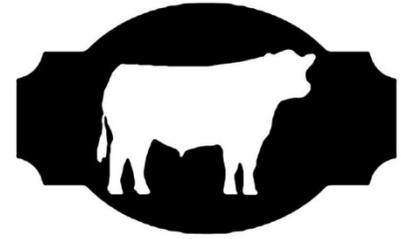
Project Start Date: September 2016

Project Completion Date: October 2019

Project status: Unfortunately, we were unable to secure additional funding to complete this project as originally envisioned. Therefore, we will be reworking the objectives for this grant to fit within this budget. These objectives will include examination of soil conditioning (alteration of soils to further its own reproduction and growth at the expense of other species) by crested wheatgrass and competition between crested wheatgrass and common rangeland forbs that serve as important food sources for sage-grouse. There is research showing soil conditioning by crested wheatgrass, however, no one has applied this research to the potential for re-establishing forbs in crested wheatgrass seedings for diversification. Progress on these objectives can be met using greenhouse experiments with undergraduate researchers. We are collecting seeds, soil collection locations, and hope to begin trials as early as Spring 2019. In the mean time, presentations and publications regarding the legacies of crested wheatgrass seedings and potential methods in diversification continue.



Oregon Beef Council Report



Beef Cattle Sciences

Progress Reports – Rangeland Ecology and Management ¹

Targeted Grazing as a Management Opportunity for Control of *Venttenata dubia* in Oregon Meadows

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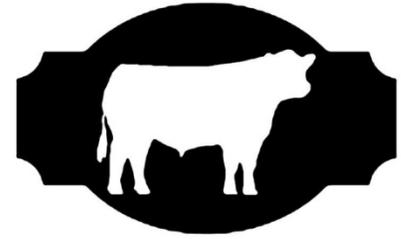
Project Objectives: *Venttenata (Venttenata dubia)* has been identified as a problem across the Pacific Northwest Region including most of Oregon. Livestock by producers, managers, and researchers around the region report *venttenata* is commonly rejected by cattle. Reasons for low utilization of *venttenata* are unclear. Both low palatability and timing of plant growth during grazing have been hypothesized as reasons for low use. However, there are no published reports on the forage quality, digestibility and potential utilization of *venttenata*. Therefore, our objectives are to document the forage quality and digestibility of *venttenata* across its growth stages.

Project Start Date: Summer 2018

Project Completion Date: October 2019

Project status: We conducted systematic sampling of *venttenata* in a mesic meadow environment within the Great Basin of southern Oregon. We evaluated the forage quality (crude protein, acid detergent fiber, neutral detergent fiber, lignin, and macro minerals: calcium, phosphorus, magnesium, potassium and sodium) and biomass production of *Venttenata* over the growing season and phases of its plant growth (April-July). We found that over the growing season, *venttenata* wet biomass ranged from 27 to 2,452 kilograms / hectare, in comparison with other vegetation in the meadow that ranged from 262 to 3,859 kilograms / hectare. Foliar cover of *Venttenata* ranged from 25% to 100%. At one hundred percent foliar cover, during the peak of forage quality *Venttenata* dry weight ranged from 180 to 447 kilograms per hectare. Forage quality peaked in late May at the elongation phase and was adequate for spring calving beef cow/calf grazing from the onset of growth in April until the last week of June. The results of this first part of our study will be presented at the Society for Range Management Meeting in February 2019. This study provides the first ever forage quality calendar for *venttenata* and will be

foundational information for exploring the grazing potential of this invasive annual grass. Our work on digestibility and utilization will continue next year.



Oregon Beef Council Report

Progress Reports – Rangeland Ecology and Management ¹

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

Contact Person: Mylen Bohle, OSU Crook County Extension Service

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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 soil fertility enhancing products claim that after being applied the first year and with continued annual or biannual application, other fertility nutrient needs CAN be cut in half the second year and beyond. Some of the products make the claim they will enhance the normal fertility program.)

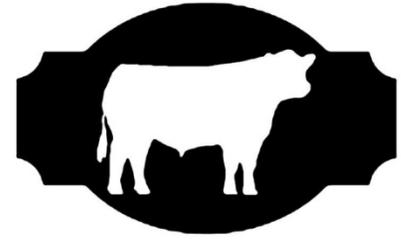
Project Start Date: Fall of 2016 (Field Work was run 2012-2015)

Project Completion Date: Fall of 2019 (could run into 2020)

Project status: Soil and Plant samples for the 4th and final year of trial “I” and 3rd and final year of trial “II” are at the Central Analytical Lab at OSU in Corvallis. I anticipate getting the 2015 soil fertility (funded by Oregon Dairy Farmers Association) and 2015 plant nutrient (funded by Oregon Beef Council) results soon (all replications were run). There were original delays because of malfunctioning machines in the lab. Those machines have been replaced. The next delay came when one of the critical nutrients (Sulfur) was not run. The problem was a miss-communication between the P.I. (me) and the lab director. I anticipate getting all of the results soon. (In the meantime, some other funding was located to run the soil fertility samples for 2012-2014 Trial “I” and 2013-2014 trial “II”. These samples are presently at the lab. Replications were combined to keep the cost down.) There are a total of 30 different soil fertility treatments. Once the data are received; it will need to be organized, run statistical analysis, tabulated and written up. More funding from Oregon Beef Council or Oregon Dairy Farmers has not been sought, until this portion of the project is completed. We still need to run quality analysis.

Preliminary Results:

Please see previous reports. We need an extension of time to complete the project.



Oregon Beef Council Report

Progress Reports – Rangeland Ecology and Management ¹

Perennial Bunchgrass Re-growth Under Different Utilization Seasons and Intensities

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Project Objectives: The objectives of this project are to test the effect of common utilization seasons and intensities on the short-term ability of native perennial bunch grasses to provide and recover visual obscenity, volume, and height by the critical sage-grouse breeding season (March 1 to June 30). Additionally, we will evaluate the cumulative effects of repeated application of common utilization regimes on the longer-term structural attributes (basal area, canopy volume) associated with individual bunchgrass plants.

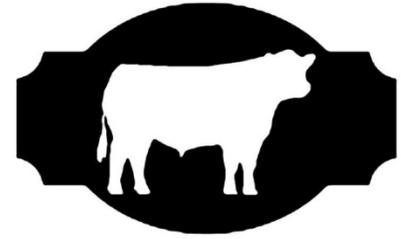
Project Start Date: Spring 2017

Project Completion Date: Fall 2020

Project status: We are conducting the study in three areas across southeast Oregon, including the Northern Great Basin Experimental Range (NGBER), Hart Mountain National Antelope Refuge, and a private ranch near Diamond, Oregon. We have established six, 5-acre study sites (i.e., blocks) at NGBER, four blocks in the Diamond study area, and three at Hart Mountain for a total of 13 blocks. All study sites are excluded from livestock use. Treatments were arranged in a randomized block design and included four different utilization treatments (none, light, moderate, and heavy) across the following four different seasons: May 1st (SPRING), May 1st and July 15th in alternating years (SPRING-DEFERRED), July 15th (DEFERRED), and November 1st (DORMANT), for a total of 12 (4 seasons X 3 utilization levels) treatment combinations and a control (no utilization). SPRING, SPRING-DEFERRED and DEFERRED treatments were all applied in spring and summer 2018 and the DORMANT treatment is planned for early November 2018. Bunchgrasses present at study sites include bluebunch wheatgrass (*Pseudoroegneria spicata*), Thurber's needlegrass (*Achnatherum thurberianum*), and Idaho fescue (*Festuca idahoensis*). Species are held constant at the site level, by selecting only the dominant species within the site. Prior to study initiation, we selected and marked three bunchgrasses for each treatment combination at each study site. In order to minimize potential effects caused by differences in grazing history at the plant or site level, all pre-selected plants were

clipped to a common stubble height of four inches during November of 2016 prior to the start of the study. In 2017- 2020, each plant will be hand clipped to the stubble height determined by our site and species specific height-weight utilization curves that correlates to its randomly assigned utilization level in order to simulate grazing. Treatments will be applied to the selected plants for four years.

Sampling: Pre-treatment sampling will occur prior to clipping (utilization) treatments on May 1st. Post-treatment sampling will occur in 2019 at multiple times during peak sage-grouse nesting season between March 1st and June 30th. The measured response variables will include: plant height, visual obstruction measured with the Robel pole method, canopy volume, and basal area.



Oregon Beef Council Report

Progress Reports – Rangeland Ecology and Management ¹

Interspace/Undercanopy Foraging Patterns of Horses in Sagebrush Habitats: Implications for Sage-Grouse

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Project Objectives: We are using a case study approach to determine the impacts of season-long (8 months/year) horse grazing on 1) sage-grouse nesting habitat structure and composition and (potentially) 2) behavioral interactions between nesting sage-grouse and grazing horses within active nesting habitat located near a water source.

Project Start Date: May of 2018

Project Completion Date: May 2022

Project status: An approximately 2,000 acre pasture has been fenced and excluded from grazing by livestock. In addition, due to infrastructure challenges we modified the experimental design. This will result in a longer study but will generate comparable data. Briefly, instead of having 2 separate pastures and collecting vegetation, horse grazing behavior, and sage-grouse nesting locations

Vegetation Sampling: All vegetation measurements will take place in June of each year of the study. Pre-treatment measurements began in 2018. The north and south halves of the pasture were split into three north/south bands that represent increasing distance from water.

Sage-Grouse: Preliminary sage-grouse nesting data has been collected in the study area for almost 10 years. We captured additional grouse the spring of 2018 and placed additional sage-grouse tracking collars on them. This practice will continue for the duration of the study.

Horse Grazing: We anticipate beginning horse grazing in 2020 or 2021 depending on the quality of preliminary data collected. We currently plan on using approximately 1 horse/100 acres from April through November. This stocking rate will be based on horse density in the nearest HMA (South

Steens). Horses will be unmanaged during the grazing period to replicate feral horse grazing. A perennial drainage on the east end of the plots will provide water for horses.

Expected outcomes/products: This research will result in first-of-its-kind data that can be used to characterize the magnitude and nature of the effects of horse grazing on nesting habitat attributes important to sage-grouse and, potentially, the influence of horse grazing on sage-grouse nesting behavior and nest success. These outcomes would be the basis for two peer reviewed journal publications.

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
<i>Rangeland Ecology and Management</i>			
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
<i>Animal Sciences</i>			
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
<i>Rangeland Ecology and Management</i>			
Wolf impact on cattle productivity and behavior (cont.)	D. E. Johnson		X
Rangeland vegetation and sediment monitoring	L. Larson	X	X
<i>Animal Sciences</i>			
Late gestation protein supplementation of beef cows	D. Bohnert		X
Grazing options with <i>Brassic</i> as 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
<i>Rangeland Ecology and Management</i>			
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
<i>Animal Sciences</i>			
PFG2 α 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
<i>Rangeland Ecology and Management</i>			
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
<i>Animal Sciences</i>			
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
<i>Rangeland Ecology and Management</i>			
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
<i>Animal Sciences</i>			
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
<i>Rangeland Ecology and Management</i>			
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
<i>Animal Sciences</i>			
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
<i>Rangeland Ecology and Management</i>			
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
<i>Animal Sciences</i>			
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
<i>Rangeland Ecology and Management</i>			
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	X
<i>Animal Sciences</i>			
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
<i>Rangeland Ecology and Management</i>			
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
<i>Animal Sciences</i>			
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
<i>Rangeland Ecology and Management</i>			
Preventing juniper reestablishment into sagebrush communities	C. Ochoa	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
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	
Annual warm season grasses for forages	G. Wang	X	X
<i>Animal Sciences</i>			
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	X
Feeding Se-fertilized hay to reduce parasite load in beef calves	J. Hall	X	X
Evaluation of biological deterrents to manage wolf movements	M. Udel	X	X

Projects funded in 2017 – 2018 FY

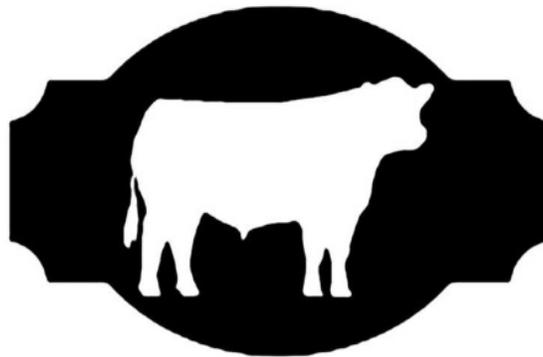
Abbreviated Project Title	Senior Investigator	Report Status	
		Progress	Full
<i>Rangeland Ecology and Management</i>			
Preventing juniper reestablishment into sagebrush communities	C. Ochoa	X	X
Conservation measures to restore rangeland on sage-grouse habitat	S. Arispe	X	
How much water do mature and juvenile juniper trees need?	R. Mata-Gonzales	X	X
Evaluation of stubble height relationship to riparian health and function	B. Endress	X	X
<i>Animal Sciences</i>			
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	X
Increasing milk production in bovine mammary cells	M. Bionaz		X
Use of platelet rich plasma for endometritis in beef heifers	M. Kutzler	X	X
<i>Out of Cycle Project</i>			
Identification of cyanobacterium in Lake county	T. Dreher	X	X

Projects funded in 2018 – 2019 FY

Abbreviated Project Title	Senior Investigator	Report Status	
		Progress	Full
<i>Rangeland Ecology and Management</i>			
Interspace/Undercanopy foraging by horses in sagebrush habitats	D. Bohnert	X	
Targeted grazing for control of ventenata dubia in OR meadows	L. Morris	X	
Conservation measures to restore rangeland on sage-grouse habitat	S. Arispe	X	
Perennial Bunchgrass re-growth under different utilization strategies	D. Johnson	X	
Preventing juniper reestablishment into sagebrush communities	C. Ochoa		X
<i>Animal Sciences</i>			
Genomic testing for prod.& perf. traits in crossbreed angus cattle	M. Kutzler	X	



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Beef Cattle Sciences

