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Beef Cattle Sciences

Oregon Beef Council Report

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Beef Cattle Sciences

Oregon Beef Council Report

Yeast culture supplementation improves feed consumption in cattle [1]

Gerd Bobe [2]

Synopsis

Yeast culture supplementation may improve feed consumption in cows around calving by decreasing cortisol secretion.

Summary

The objective of this study was to evaluate if and how feeding of *Saccharomyces cerevisiae* fermentation product (Diamond V Original XP™) affects feed consumption and energy status around calving. Multiparous Holstein cows were given individually a supplement containing either 0 (control: n = 14), 56 (n = 15), or 112 grams (n = 13) of XP daily during morning lock-up as a top dressing to their ration. The supplement consisted of 0, 56, or 112 grams of XP mixed with 84 grams of molasses and 168, 112, or 56 grams of corn meal, respectively. Supplement feeding began four weeks before predicted calving date (at least 14 days before calving) and ended 28 days after calving. Blood samples were collected at days -7, -3, -1, 0, 1, 3, and 7 after calving to measure serum concentrations of cortisol, insulin, haptoglobin, serum amyloid A, visfatin, glucose, insulin, non-esterified fatty acids, β-hydroxybutyrate, and urea N. Feeding XP increased supplement intake at the day of calving (both XP dosages combined versus control; P = 0.03) and decreased serum cortisol concentrations during the two weeks before and after calving (P = 0.006). Our results suggest that yeast culture supplementation may improve feed consumption around calving by decreasing cortisol secretion.

Introduction

The time around calving and after shipping are the most stressful time periods in the lives of dairy and beef cattle, respectively. Cattle that eat well during these time periods stay healthy and are more likely to produce according to their genetic potential, thus guaranteeing a high profit. In contrast, cattle that do not eat well are more susceptible to diseases and produce below their genetic potential, leading to a smaller profit margin. Being sick further decreases feed intake, resulting in even greater profit losses, as approximately 10% of dairy cows die or have to be culled in the first 4 weeks postpartum (Pinedo et al, 2010). Similar losses can be observed in beef cattle after shipping. Thus, feed supplements that improve feed consumption around calving and after shipping are needed.

Saccharomyces cerevisiae fermentation product (**SCFP**; Diamond V Original XP™) contains ingredients that improve feed palatability and digestibility in beef cattle and dairy cows (Ramsing et al., 2009). Palatability is a major driving force of feed consumption; especially during times of increased stress, when feed consumption is depressed. Stress, inflammation, and adipose-associated cytokines (adipokines) are major factors that decrease feed consumption (Roche et al., 2008). Stress can be measured through cortisol, which

1. This document is part of the Oregon State University – 2012 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
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reduces feed consumption. Inflammation can be measured using the acute phase proteins haptoglobin and serum amyloid A (**SAA**; Cray et al., 2009). Visfatin is an adipose-tissue associated protein that amplifies the inflammatory response and promotes insulin secretion and sensitivity (Adeghate, 2008).

Our hypothesis was that SCFP improves around calving feed consumption, as measured by supplement consumption, and energy status, using serum concentrations of glucose, insulin, non-esterified fatty acids (**NEFA**), and β-hydroxybutyrate (**BHBA**) as indicators, by decreasing serum concentrations of cortisol, acute phase proteins, and/or visfatin, leading to a greater profit margin. The objective of the study was to examine if and how SCFP may improve feed consumption and energy status around calving.

Materials and Methods

The study was conducted on a 1,000-head commercial dairy farm in Oregon's Central Willamette Valley. The study design was a randomized complete block design. Holstein cows were blocked by upcoming parity (2, 3, 4 or greater) and randomly assigned to three treatment groups. The treatment consisted of 0 (**control**; n = 14), 56 (n = 15), or 112 grams per day (n = 13) of XP (Diamond V Original XP™; Cedar Rapids, IA) mixed with 84 grams of molasses and 168, 112, or 56 grams of corn meal, respectively. The XP consisted of fermentation metabolites produced from *Saccharomyces cerevisiae* when grown using a proprietary technique, along with the fermentation medium (ground yellow corn, hominy feed, corn gluten feed, wheat middlings, rye middlings, diastatic malt and corn syrup, and cane molasses) used in the production process. The supplement was top-dressed individually to each cow during the morning feeding, when cows were locked in head stanchion lock-ups for a period of 30 to 45 minutes. The treatment period started four weeks before the expected calving date and ended four weeks after calving.

During the last four weeks before expected calving, cows were housed in a group of 40 to 50 cows in a straw-bedded free stall barn. Cows were fed once in the morning (7:30) a typical dry-cow total mixed ration (**TMR**), which was formulated according to NRC guidelines (NRC, 2001). After calving, cows were moved to the hospital pen that housed 30 to 40 cows. The hospital pen was used for fresh cows (first two days postpartum) and cows that needed major medical treatment including antibiotic treatment requiring the milk to be discarded, oral or intravenous treatment with glucose precursors, or both. Cows from the hospital pen were fed once in the morning (7:00) a typical fresh cow TMR, which was formulated according to NRC guidelines (NRC, 2001) and were milked twice a day. The other cows in the first month of lactation were grouped in an early lactation pen that housed 90 to 110 cows. Cows from the early lactation pen were fed once in the morning (9:00) the same TMR as the cows in the hospital pen and were milked six times a day.

Supplement consumption was monitored daily and recorded as no, partial, or complete consumption. In addition, 5 to 8-mL blood samples were taken at days --7 (-10 to -5), -3 (-4 or -3), -1 (-2 or -1), 0, 1, 3, and 7 postpartum and analyzed for serum concentrations of cortisol (bovine specific ELISA; Endocrine Technologies, Newark, CA), haptoglobin (bovine specific ELISA; Life Diagnostics, Inc., West Chester, PA), SAA (multispecies ELISA; Life Technologies, Grand Island, NY), visfatin (human visfatin C-terminal enzyme immunoassay; Phoenix Pharmaceuticals Inc., Birmingham, CA; human and bovine visfatin have 98% homology; Adeghate, 2008), glucose (Stanbio Laboratory; Boerne, TX), insulin (bovine specific ELISA; Endocrine Technologies), non-esterified fatty acids (**NEFA**; Stanbio Laboratory), β-hydroxybutyrate (**BHBA**; Stanbio Laboratory), and urea N (Stanbio Laboratory). Economic data were calculated as follows: total costs were calculated as the sum of incurred expenses for XP, medical treatment, and milk profit lost due to discarded milk and culling. Total income was calculated from milk and cow sales. The difference between incurred costs and income was defined as net gain. Because we could not measure feed intake or hours of labor, general feed and labor costs were not included in the calculation.

Serum data were analyzed as repeated measures randomized block design using the PROC MIXED procedure of SAS (SAS Institute, 2001). To achieve normal distribution for their serum concentrations, concentrations of SAA, visfatin, glucose, and NEFA were ln-transformed and concentrations of cortisol, haptoglobin, insulin, and BHBA were twice ln-transformed. The statistical model included as fixed effects: SCFP supplementation (0, 56, or 112 grams per day of XP), sampling time (day -7, -3, -1, 0, 1, 3, and 7 postpartum), parity (2, 3, 4 or greater), and the

interaction between XP supplementation and sampling time. The variance-covariance structure of repeated measures within cow was modeled using the heterogeneous first-order autoregressive variance-covariance matrix. Values presented in the figures and tables are least-squares means (**LSM**) and their standard errors (**SEM**) that are transformed back to their original measurement scale. Economic data were analyzed using PROC GLM. Feed consumption data were analyzed using PROC GLIMMIX as % complete consumption assuming a binomial distribution. In both models, SCFP supplementation and parity were the fixed effects. All statistical tests were two-sided. Orthogonal contrasts were constructed to estimate the effect of XP supplementation (both XP doses combined versus control) and XP dose (112 versus 56 grams per day) using the ESTIMATE statement. Significance was declared at $P \leq 0.05$ and a tendency at 0.05 to 0.15.

Results

Feeding SCFP increased supplement intake at the day of calving (% complete consumption control: 53 ± 16%; 56 g/d XP: 82 ± 11%; 112 g/d XP: 94 ± 7%; both XP doses combined versus control: $P = 0.03$; Figure 1). By contrast, no significant differences in supplement intake between treatment groups were observed in the week before or after calving (results not shown). We could not measure feed intake in this study; however, feeding XP during the transition period generally increases feed intake by approximately 2 to 5% (Robinson, 1997; Robinson and Garrett, 1999; Dann et al., 2000; Wang et al., 2001; Erasmus et al., 2005; Ramsing et al., 2009). The highly palatable taste of SCFP may specifically promote feed intake at times when intake is low such as around calving (Robinson, 1997; Robinson and Garrett, 1999; Ramsing et al., 2009) or after transportation. The beneficial effect of XP may be independent of its taste and caloric content, because the supplements contained equal, high concentrations of molasses (33% of total supplement) and the amount of ground corn was adjusted to substitute for the amount of XP fed.

Feeding SCFP decreased serum concentrations of cortisol in the two weeks before and after calving (XP versus control: $P = 0.006$; Figure 2). Little is known the biological mechanisms by which SCFP may promote feed intake at times when intake is low. The sweet smell and the highly palatable taste of SCFP may reduce the secretion of stress hormones, including cortisol, and, thereby improve feed intake.

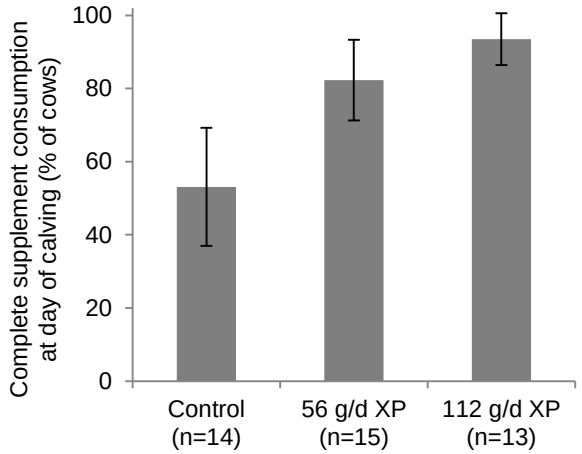

Figure 1. Effect of feeding multiparous dairy cows *Saccharomyces cerevisiae* fermentation product (XP) during the last four weeks before and the first four weeks after calving on complete supplement consumption at the day of calving (LSM ± SEM). Feeding XP increased supplement intake at the day of calving (both XP dosages combined versus control; $P = 0.03$).

The effect of feeding SCFP on serum concentrations of SAA differed between before and after calving (Table 1). Before calving, feeding SCFP tended to decrease serum concentrations of SAA (XP versus control: $P = 0.09$) and at greater dosages serum concentrations of haptoglobin (112 versus 56 g/d XP: $P = 0.07$). The yeast fermentation metabolites include antioxidants, vitamins, soluble fiber, and other bioactive compounds that have anti-inflammatory properties and reduce cytokine release (Jensen et al., 2008). After calving, feeding SCFP, however, increased serum concentrations of SAA (XP versus control: $P = 0.04$), which have been also reported previously in beef-lot steers (Emmanuel et al., 2007). Further studies are warranted to clarify the effect of SCFP on acute phase response and inflammation.

Feeding SCFP tended to increase serum concentrations of urea N at calving (XP versus control: $P = 0.07$) and at greater concentrations tended to decrease BHBA concentrations in the last week prepartum (112 versus 56 g/d XP: $P = 0.07$; Table 1). The increase in serum urea N may be associated with increased protein deamination for gluconeogenesis or as energy precursor. An alternative explanation is that SCFP may increase protein intake, as reported by Robinson and Garrett (1999).

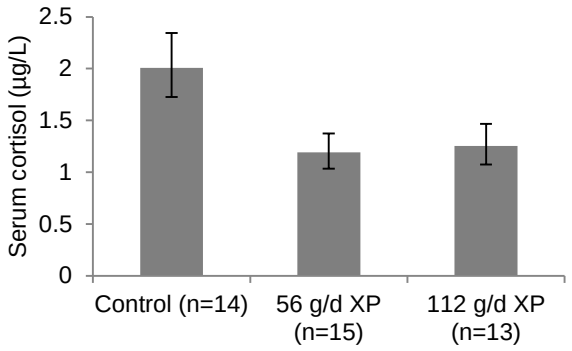

Figure 2. Effect of feeding multiparous dairy cows *Saccharomyces cerevisiae* fermentation product (XP) during the last four weeks before and the first four weeks after calving on serum cortisol concentrations during the two weeks around calving (LSM ± SEM). Feeding XP decreased serum cortisol concentrations (both XP dosages combined versus control; $P = 0.006$).

Feeding SCFP tended to increase daily income in the first 4 weeks after calving (control: U.S.$ 15.8; 56 g/d XP: $ 17.3; 112 g/d XP: $ 18.2; SEM = 1.1; 2XP versus control: $P = 0.11$) but did not result in statistical significant differences in daily net gain (control: $ 11.7; 56 g/d XP: $ 11.4; 112 g/d XP: $ 13.1; SEM = 2.6; XP versus control: $P = 0.85$; Figure 3). Although not statically significant, the 12% greater net profits after feeding 112 grams per day of XP may provide farmers sufficient incentive to supplement with SCFP.

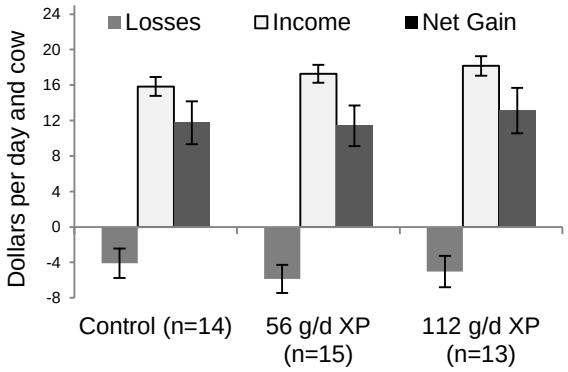

Figure 3. Effect of feeding multiparous dairy cows *Saccharomyces cerevisiae* fermentation product (XP) during the last four weeks before and the first four weeks after calving on economic data for the first four week postpartum (LSM ± SEM). Feeding XP tended to increase income (both XP dosages combined versus control; $P = 0.13$).

Conclusions

In conclusion, feeding SCFP may improve feed consumption around calving and other stressful events such as transportation by decreasing secretion of the stress hormone cortisol. The effect of feeding SCFP on inflammatory indicators differed between before (anti-inflammatory) and after calving (pro-inflammatory) and requires further investigation. Feeding 112 grams per day of SCFP may result in modest but not statistically significant increases in net profit (approximately 12%).

Acknowledgements

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Table 1. Effect of feeding multiparous dairy cows *Saccharomyces cerevisiae* fermentation product during the last four weeks before and the first four weeks after calving on serum indicator concentrations during the last week before calving (prepartum), at calving, and the first week after calving (postpartum)[1]

Indicator	Treatment (LS Means ± SEM)			P-value of Contrasts[2]	
	0 g/d XP	56 g/d XP	112 g/d XP	Control vs. XP	56 vs. 112 g/d XP
	N = 14	N = 15	N = 13		
Serum Amyloid A (mg/L)					
Prepartum	23.7 ± 5.9	14.5 ± 3.6	14.1 ± 3.8	0.09	0.94
Calving	71.7 ± 20	99.1 ± 27	37.9 ± 14	0.55	0.49
Postpartum	84.2 ± 26	178 ± 54	187 ± 61	0.04	0.91
Haptoglobin (mg/L)					
Prepartum	8.74 ± 1.2	9.22 ± 1.3	6.62 ± 0.9	0.45	0.07
Calving	22.1 ± 7.0	37.9 ± 14	17.5 ± 5.4	0.73	0.09
Postpartum	77.4 ± 28	144 ± 57	113 ± 46	0.25	0.65
Urea N (mg/dL)					
Prepartum	10.5 ± 0.8	11.6 ± 0.8	11.8 ± 0.9	0.21	0.88
Calving	13.2 ± 1.3	16.5 ± 1.2	15.4 ± 1.3	0.07	0.55
Postpartum	12.2 ± 0.9	12.7 ± 0.9	13.1 ± 1.0	0.56	0.76
BHBA (mMol)					
Prepartum	0.56 ± 0.04	0.59 ± 0.04	0.50 ± 0.04	0.58	0.07
Calving	0.76 ± 0.07	0.72 ± 0.06	0.66 ± 0.07	0.38	0.45
Postpartum	0.85 ± 0.07	0.79 ± 0.06	0.93 ± 0.08	0.98	0.15
NEFA (µEq/L)					
Prepartum	353 ± 47	439 ± 59	355 ± 52	0.49	0.27
Calving	473 ± 68	463 ± 65	411 ± 62	0.64	0.55
Postpartum	746 ± 96	689 ± 85	602 ± 81	0.34	0.79
Glucose (mg/dL)					
Prepartum	68.7 ± 1.8	65.3 ± 1.8	68.8 ± 2.2	0.45	0.19
Calving	71.5 ± 4.0	77.9 ± 4.4	79.9 ± 4.7	0.16	0.75
Postpartum	61.6 ± 2.9	63.7 ± 3.0	62.7 ± 3.1	0.65	0.82
Insulin (µg/L)					
Prepartum	0.18 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	0.71	0.86
Calving	0.20 ± 0.05	0.20 ± 0.05	0.24 ± 0.06	0.80	0.59
Postpartum	0.18 ± 0.02	0.20 ± 0.03	0.19 ± 0.03	0.61	0.87
Visfatin (µg/L):					
Prepartum	6.15 ± 0.52	6.54 ± 0.54	6.22 ± 0.56	0.71	0.67
Calving	6.22 ± 0.52	6.46 ± 0.52	6.16 ± 0.54	0.88	0.68
Postpartum	7.05 ± 0.59	7.20 ± 0.58	6.88 ± 0.60	0.99	0.69

[1]Least square means and standard error of mean
[2]Probabilities of orthogonal contrasts

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Beef Cattle Sciences

Oregon Beef Council Report

Can prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) be used during the postpartum anestrus period to improve uterine health and reproductive efficiency? [1]

Adrienne M. Lulay [2], Matthew J. Cannon [2] and Alfred R. Menino, Jr. [2]

Synopsis

Administration of Lutalyse, the pharmaceutical preparation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), to postpartum dairy cows increases uterine neutrophils, a subset of white blood cells, and decreases uterine pathogenic bacteria, days open and services per conception.

Summary

The objective of this research was to determine whether administration of Lutalyse in the early postpartum period has a positive impact on uterine health and reproductive efficiency. Evaluation of the Lutalyse injection protocol on uterine health and reproductive efficiency was conducted at the Oregon State University Dairy Research Center (OSUDRC) and a private cooperating dairy, respectively. Cows at both sites were divided into four treatments. Treatments consisted of two injections im of: 1) saline (5 ml) on Days 0-1 and 14 postpartum (where Day 0 = day of calving), 2) saline (5 ml) on Days 14 and 28 postpartum, 3) Lutalyse (25 mg/5 ml) on Days 0-1 and 14 postpartum and 4) Lutalyse (25 mg/5 ml) on Days 14 and 28 postpartum.

For all treatments at the OSUDRC, on the day of the second injection (Day 14 or 28; Time 0) and 24 h after the second injection (Time 24), the cow's uterus was sampled for uterine bacterial load using a double guarded swab. The swab was placed into a tube containing 10 ml of DPBS and transported to the laboratory within 1 h of collection for bacterial culture. To provide an assessment of the uterine neutrophil population, a guarded CytoBrush was passed into the uterus immediately following the uterine swabbing at Times 0 and 24. A cell smear was prepared from the CytoBrush and neutrophils were counted. Blood samples were drawn at Times 0 and 24 to validate plasma progesterone concentrations.

Starting at Day 50 postpartum, cows at the private, cooperating dairy were administered two 25-mg injections im of Lutalyse two weeks apart as an estrous pre-synchronization program. After the second injection, estrus was monitored twice daily and determined based on the removal of tail chalk applied using paint sticks (All-Weather Paintstik; LA-CO, Chicago IL). When estrus was observed, cows were artificially inseminated 12 h later. Cows not observed in estrus within 2 weeks were started on an Ovsynch timed artificial insemination program consisting of: 2 ml of GnRH (gonadorelin diacetate tetrahydrate; 100 µg; Cystorelin; Merial LLC, Duluth, GA) on Day 0 im, 5 ml of Lutalyse on Day 7 im, 2 ml of GnRH on Day 10 im and timed artificial insemination 24 h after the second GnRH injection. All cows remaining on the farm until 100 days postpartum were artificially inseminated the

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first time between 65 and 91 days postpartum. Pregnancy status was monitored by rectal palpation by a veterinarian at 6, 17 and 29 weeks post-insemination. Cows were injected with 2 ml of GnRH im 14 days before the scheduled pregnancy diagnosis so the Ovsynch program described earlier could be started immediately if a cow was diagnosed as not pregnant. If a cow was observed in estrus after a confirmed pregnancy diagnosis, she was re-examined and if not pregnant recorded as aborted, and re-bred on the next scheduled Ovsynch program. At 34 weeks postpartum, cows were moved to a pen containing two bulls for breeding by natural service.

Introduction

Uterine infections during the period after calving, or the postpartum period, are a significant problem to dairy producers. Dairy cows are particularly susceptible to develop uterine infections because of conditions associated with housing and production. Bacterial infections, such as endometritis, metritis and pyometra, occurring within 28 days postpartum affect 10 to 50% of cows annually. The majority of these infections go undiagnosed and untreated. Fertility is reduced in cows with uterine infections because of abnormal reproductive cycles, lower conception rates (20% lower than healthy cows), and longer days open (an average of 30 days longer than healthy cows). The cost of days open beyond 100 days has been estimated to be as much as $5.40/day for the dairy cow. Uterine infections in dairy cattle can therefore cost producers as much as $162 ($5.40/day x 30 days) per affected cow. Depending on the uterine infection incidence one uses to calculate cost to Oregon dairy producers on a state-wide basis, income loss due to uterine infections is estimated to range between $2 million (125,000 Oregon dairy cows x 10% x $162/infected cow) to as much as $10.1 million (125,000 cows x 50% x $162/cow) annually.

During pregnancy, the uterus is a sterile environment, but during calving and the early postpartum period, bacteria from the cow's environment can colonize the uterus, causing infection (Singh et al., 2008). Fertility is impaired in infected cows due to disruption of normal physiological events in the uterus and ovary. It is known that susceptibility of the uterus to bacterial infection is higher when the ovarian hormone progesterone is high, and that susceptibility is lower when the ovarian hormone estrogen and the uterine hormone prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are high (Williams et al., 2007; Kaneko and Kawakami, 2009). Significant research effort has also been directed towards studying the pathogenic microorganisms that cause uterine infections and the effects these microorganisms have on impairment of fertility in cattle. However, despite this research, preventative practices and new treatment strategies to alleviate the problem have not been developed, and the incidence of uterine infection has not changed significantly in cattle over the previous 30 years. New knowledge in this area is a necessary first step towards development of new and practical methods for preventing uterine infections and novel therapeutic regimes not requiring antibiotics for treatment of uterine infections in cattle. Therefore, the objective of this research was to evaluate the effects of a Lutalyse injection protocol, the pharmaceutical preparation of $PGF_{2\alpha}$, on uterine bacterial and neutrophil numbers, days open and services per conception in postpartum dairy cows.

Materials and Methods

Animals

The effects of the Lutalyse injection protocol on uterine health and reproductive efficiency was evaluated at the Oregon State University Dairy Research Center (OSUDRC) and a private cooperating dairy, respectively. Twenty-eight and 64 postpartum cows at the OSUDRC and the cooperating dairy, respectively, were randomly assigned to four treatments. Treatments consisted of two injections im of: 1) saline (5 ml) on Days 0-1 and 14 postpartum (where Day 0 = day of calving), 2) saline (5 ml) on Days 14 and 28 postpartum, 3) Lutalyse (Pfizer, New York, NY; 25 mg/5 ml) on Days 0-1 and 14 postpartum and 4) Lutalyse (25 mg/5 ml) on Days 14 and 28 postpartum. All work conducted with animals in this research was approved by the Oregon State University Institutional Animal Care and Use Committee.

Effects of Lutalyse treatment on uterine bacterial and neutrophil populations in postpartum dairy cows

Sampling for uterine bacterial populations

For all treatments at the OSUDRC, on the day of the second injection (Day 14 or 28), the cow's uterus was sampled for uterine bacterial load using a double guarded swab (Time 0). The swab was placed into a tube containing 10 ml of Dulbecco's phosphate buffered saline (DPBS) and transported to the laboratory within 1 h of collection for culture.

Twenty-four hours after the first swab, a second uterine swab was collected (Time 24), placed into a tube containing 10 ml of DPBS and transported to the laboratory within 1 h of collection for culture.

Sampling to assess neutrophil populations

To provide an assessment of the uterine neutrophil population, a subset of white blood cells, a guarded CytoBrush was passed into the uterus immediately following the uterine swabbing at Times 0 and 24. The CytoBrush was rolled onto a microscope slide to create a cell smear. The smear was stained using the Wright/Giemsa staining procedure for assessing white blood cell populations and neutrophils were counted. Cows received their second injection following completion of the uterine swabbing and CytoBrush procedures at Time 0.

Bacterial cultures

Serial dilutions using DPBS as the diluting fluid (0, 1/10 and 1/100) were made from the original uterine sample and 100 µl was plated onto each side of a Blood Agar/MacConkey's Agar bacteriological culture biplate. Biplates were incubated for 1 h at room temperature, transferred to a 37°C incubator, turned over and cultured with the lid side down on the incubator tray for 24 h. Following incubation at 37°C bacterial colonies growing on each agar type were counted. Colony counts on the blood agar side provided the total number of pathogenic bacteria and counts from the MacConkey's agar side, a selective medium, provided the number of *E. coli*.

Progesterone analysis

Blood samples (10 ml) were drawn by coccygeal venipuncture at Times 0 and 24 to validate plasma progesterone concentrations. Blood was collected into Vacutainer tubes containing sodium heparin as the anti-coagulant. Plasma was separated by centrifugation at 1000xg for 10 min at 4°C and stored at -20°C until assayed for progesterone. Progesterone was assayed using kits purchased from CALBIOTECH (Spring Valley, CA).

Effects of Lutalyse treatment on days open and services per conception in postpartum dairy cows

Cows were managed at the cooperating dairy under the following standard operating procedures. Cows were housed in free stalls and received *ad libitum* water and a TMR formulated to meet the NRC recommended diet for high producing cows. Starting at Day 50 postpartum, cows were administered two 25-mg injections im of Lutalyse two weeks apart as an estrous pre-synchronization program. After the second injection, estrus was monitored twice daily and determined based on the removal of tail chalk applied using paint sticks (All-Weather Paintstik; LA-CO, Chicago IL). When estrus was observed, cows were artificially inseminated 12 h later. Cows not observed in estrus within 2 weeks were started on an Ovsynch timed artificial insemination program consisting of: 2 ml of GnRH (gonadorelin diacetate tetrahydrate; 100 µg; Cystorelin; Merial LLC, Duluth, GA) on Day 0 im, 5 ml of Lutalyse on Day 7 im, 2 ml of GnRH on Day 10 im and timed artificial insemination 24 h after the second GnRH injection. All cows remaining on the farm until 100 days postpartum were artificially inseminated the first time between 65 and 91 days postpartum.

Pregnancy status was monitored by rectal palpation by a veterinarian at 6, 17 and 29 weeks post-insemination. Cows were injected with 2 ml of GnRH im 14 days before the scheduled pregnancy diagnosis so the Ovsynch program described earlier could be started immediately if a cow was diagnosed as not pregnant. If a cow was observed in estrus after a confirmed pregnancy diagnosis, she was re-examined and if not pregnant recorded as aborted, and re-bred on the next scheduled Ovsynch program. At 34 weeks postpartum, cows were moved to a pen containing two bulls for breeding by natural service.

Statistical analyses

Differences between Times 0 and 24 in the total numbers of bacteria and neutrophils, and plasma progesterone concentrations, and differences between days open and services to conception due to treatment were analyzed by analysis of variance (ANOVA) for a 2 x 2 factorial design. Sources of variation in the ANOVA included treatment regimen (Days 0-1 and 14, Days 14 and 28), injection (saline, Lutalyse) and the treatment x injection interaction. If significant effects were observed in the ANOVA, differences between means were evaluated by Fisher's least significant differences procedures. All analyses were performed using the NCSS statistical software program (Number Cruncher Statistical System; 2000, Jerry Hintze, Kaysville, UT).

Results

Effects of Lutalyse treatment on uterine neutrophil and bacterial populations in postpartum dairy cows

Lutalyse treatment increased (P<0.05) the number of uterine neutrophils between the 0 and 24 h samplings compared to cows injected with saline (23.4 ± 5.5 vs. -2.5 ± 9.7 cells, respectively). Interval and the treatment x interval interaction were not significant sources of variation in number of neutrophils (Figure 1). Conversely, Lutalyse treatment decreased (P<0.05) total bacteria present in the uterus compared to cows injected with saline (-1300 ± 843 vs. 1127 ± 526 cells, respectively). Interval and the treatment x interval interaction were not significant sources of variation for total uterine bacteria (Figure 2). Bacterial colony growth on MacConkey's agar, presumably *E. coli*, was infrequent in the cows sampled hence no analyses were conducted.

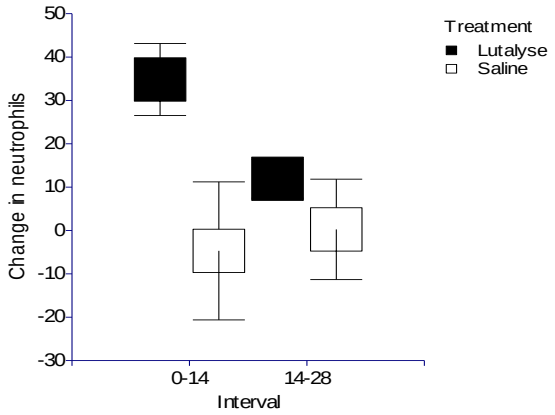

Figure 1. Changes in uterine neutrophils in cows injected with Lutalyse or saline Days 0-14 or 14-28 postpartum.

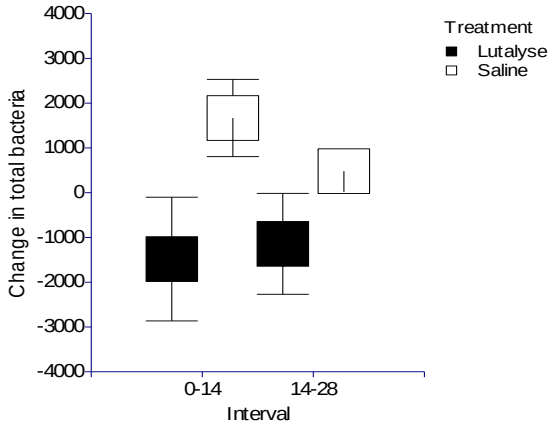

Figure 2. Changes in total uterine bacteria in cows injected with Lutalyse or saline Days 0-14 or 14-28 postpartum.

Cows injected with Lutalyse experienced reduced plasma progesterone concentrations compared to cows injected with saline (-2.0 ± 1.1 vs. 0.2 ± 0.4 ng/ml, respectively) however no significant differences were observed. Plasma progesterone concentration decreased (P<0.05) in cows injected during the 14-28 compared to the 0-14 interval (-2.1 ± 1.1 vs. 0.4 ± 0.1 ng/ml, respectively) and the treatment x interval interaction was a significant source of variation (Figure 3).

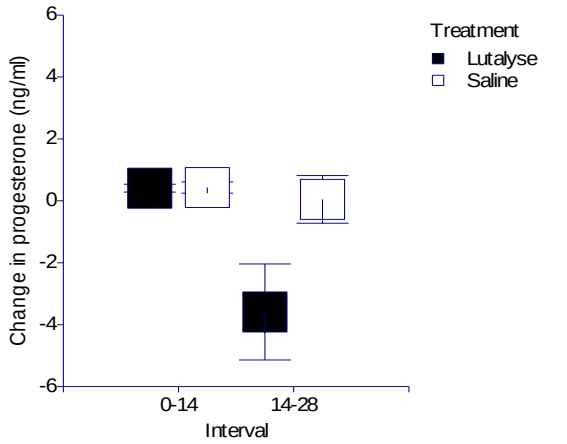

Figure 3. Changes in plasma progesterone in cows injected with Lutalyse or saline Days 0-14 or 14-28 postpartum. [a,b] Means without similar superscripts differ (P<0.05).

Effects of Lutalyse treatment on days open and services per conception in postpartum dairy cows

Of the 64 cows initially committed to this experiment, seven were not pregnant at the conclusion of the study hence data were recovered from 57 cows. Lutalyse treatment reduced (P<0.05) the number of days open compared to saline (Figure 4). Interval (127.3 ± 11.5 vs. 146.6 ± 11.8 days for 0-14 vs. 14-28 days postpartum, respectively) and the treatment x interval interaction were not significant factors in reducing days open (Table 1). Likewise, Lutalyse treatment reduced (P<0.10) the number of services per conception compared to saline (Figure 5). Interval (2.3 ± 0.3 vs. 3.0 ± 0.3 services for 0-14 vs. 14-28 days postpartum, respectively) and the treatment x interval interaction were not significant sources of variation in services per conception (Table 1).

Conclusions

Results from the current experiments suggest $PGF_{2\alpha}$ exerts direct effects on neutrophil proliferation and/or release. Lutalyse treatment

increased uterine neutrophils and decreased total uterine bacteria 24 h after injection. Administering exogenous $PGF_{2\alpha}$ may induce greater neutrophil release from the bone marrow, thereby making more neutrophils available to enter the uterus. The reduction in bacteria 24 h after Lutalyse injection corresponds well with the increase in uterine neutrophils. Beneficial effects of $PGF_{2\alpha}$ were also observed on reproductive performance of cows receiving treatment at the commercial dairy. Cows receiving the two injection Lutalyse protocol had reduced days open and number of services per conception compared to saline injection. The reduction in days open was 35 days which provides an estimated savings of approximately $75 per cow. The reduction in services per conception was about one, which can amount to approximately $10-$20 in savings on semen costs and labor for breeding and heat detection. The two Lutalyse injection protocol would definitely pay for itself if a single dose of Lutalyse is under $3/dose. Lutalyse is commonly used in dairy operations to synchronize estrous cycles for artificial insemination hence this regimen can be readily implemented with relatively little cost and effort. Treating cows with Lutalyse early in the postpartum period could provide an inexpensive method not requiring antibiotics for preventing uterine infections in cattle and improving reproductive efficiency.

Table 1. Number of days open and services per conception for dairy cows injected with Lutalyse or Saline Days 0-14 or 14-28 postpartum.

	Days open (n)		Services per conception (n)	
Treatment	0-14	14-28	0-14	14-28
Lutalyse	102.6± 8.3 (12)	132.4 ± 11.3 (17)	1.8 ± 0.2 (12)	2.7 ± 0.4 (17)
Saline	145.8 ±18.0 (16)	166.6 ± 23 (12)	2.8 ± 0.5 (16)	3.3 ± 0.7 (12)

Acknowledgements

This research was generously supported by the Oregon Beef Council and the Oregon Agricultural Experiment Station.

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Figure 4. Number of days open for cows injected with Lutalyse or saline Days 0-14 or 14-28 postpartum. [a,b] Means without similar superscripts differ (P<0.05).

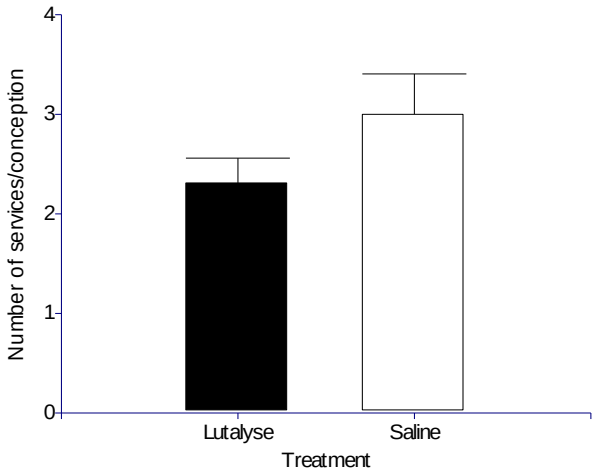

Figure 5. Number of services per conception for cows injected with Lutalyse or saline Days 0-14 or 14-28 postpartum. [a,b] Means without similar superscripts differ (P<0.10).

Oregon State University

BEEF097

Beef Cattle Sciences

Oregon Beef Council Report

Application of a plasminogen activator assay to assess bovine embryo viability during embryo transfer procedures [1]

Ruben Mendoza [2], Katie Hayes [2], and Alfred R. Menino, Jr [2]

Synopsis

Development of the proposed technique for assessing the likelihood of embryo survival following an embryo transfer would benefit commercial cattle producers by eliminating the cost associated with transferring embryos with reduced success of pregnancy establishment and maintaining non-pregnant recipients.

Summary

The specific aim of this project was to determine if the amount of plasminogen activator (PA) produced by cow embryos was related to success in establishing a pregnancy. In a preliminary experiment, mean PA production was similar (P>0.10) for embryos establishing a pregnancy compared to embryos failing to generate a pregnancy (1.3±0.5 vs. 4.6±2.8 mIU/ml/h, respectively). However, when a threshold level for PA production equal to 0.3 mIU/ml/h was established, 32% (7/22) of embryos with PA production above threshold compared to only 9% (1/11) of embryos with PA production equal to or below threshold developed into pregnancies. Although encouraging, the overall conception rate for the preliminary experiment was below average (24%) which contributed to the lack of statistical significance observed in conception rates between embryos above vs. below threshold PA production. The overall below average conception rate was attributed to the 22-h duration of culture embryos were subjected to as part of the PA assay because extended culture such as this has been shown to depress pregnancy rates. Recent experiments were designed to minimize this culture interval to improve transfer conception rates and make this procedure more applicable to on-farm conditions. If a reliable and significant relationship is identified between PA production and success of pregnancy establishment, an additional marker would be available to grade and select embryos for transfer.

Introduction

A method to accurately determine viability among embryos and provide a reasonable prediction of pregnancy rate in embryo transfer is as yet unavailable. When faced with the decision as to which embryos in a flush should be transferred to recipients and will result in the greatest number of offspring, the operator often relies on gross observations. These observations, which include such features as degenerating or irregular sized cells (blastomeres), granulation and vesicles, are useful and fairly accurate in determining embryos of exceptionally poor quality but are of little use in differentiating within the range of viability. A method for accurately assessing embryo viability

1. This document is part of the Oregon State University – 2012 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.

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prior to transfer to a recipient would afford some measure of the embryo's ability to sustain a pregnancy (Sreenan and Diskin, 1987). Such a system would provide for determining embryo quality and predicting the success of pregnancy establishment. Grading schemes based on these morphologic aspects of embryos are fairly accurate in predicting pregnancy rates between embryos which are several grades apart. The morphologic scoring system, however, is not that accurate for embryos which either grade closely or are in the fair, good and excellent grades. Attempts at grading embryos on the basis of variation in gross morphology and then evaluating pregnancy rates in females receiving various grades were conducted by Shea (1981). In this particular work, three grades of bovine embryos were defined. Grade 2 embryos were characterized by uneven blastomere size, extensive blastomere extrusion and evidence of membrane rupture. Grade 3 embryos were designated as having an average appearance and Grade 4 embryos were perfectly symmetrical, evenly granulated, lacking in blastomere extrusion and possessed no deformations in the zona pellucida. Pregnancy rates for grades 4, 3 and 2 embryos were 71, 56 and 44%, respectively. Lindner and Wright (1983) devised an alternative grading scheme based on the gross morphology of bovine embryos that was comprised of four ranks. Pregnancy rates for excellent, good, fair and poor embryos were 45, 44, 27 and 20%, respectively. Studies such as these illustrate that despite the apparent range in morphology encountered in evaluating embryos prior to transfer, pregnancy can still be established in recipient females with embryos of supposedly inferior morphology.

Our laboratory has been researching production of the protease plasminogen activator (PA) by cultured cattle embryos since 1987 (Menino and Williams, 1987). Plasminogen activator secretion is undetectable until the blastocyst stage, increases during blastocoelic expansion and initiation of hatching and remains elevated throughout and after loss of the zona pellucida. High levels of PA production were associated with embryos undergoing vigorous development whereas low levels of PA were produced by poorly developing embryos (Kaaekuahiwi and Menino, 1989). The assay used in these studies is a caseinolytic agar gel assay which is simple yet sensitive. Plasminogen activator has many roles in the early embryo and our laboratory has recently observed that this protease is involved in the establishment of the extraembryonic endoderm which is a component of the fetal membranes in the cow fetus (Singleton and Menino, 2005). Based on these observations, PA secretion by cow embryos may be used as an accurate indicator of embryo viability and, potentially, pregnancy establishment.

In a preliminary experiment, forty-seven embryos with normal morphology were collected from 10 superovulated donors and cultured for 22 h in a humidified atmosphere of 5% CO_2 in air at 39°C. Following culture, medium was recovered and embryos were graded and assigned quality scores and 33 embryos were transferred to timed recipients. Eight of 33 recipients (24%) were diagnosed pregnant. Mean PA production and post-culture quality scores for embryos generating vs. failing to generate a pregnancy did not differ. When a threshold value was set at 0.3 mIU PA/ml/h, only 1 of 11 (9%) embryos with PA production below or equal to this value produced a pregnancy whereas 7 of 22 (32%) embryos above threshold generated a pregnancy. The 11 embryos with PA production below this threshold value represented 33% (11/33) of the total number of embryos transferred.

Two aspects of this study clearly require refinement. First, the pregnancy rate of 24% is low and should be increased to approach industry standards. However, in defense of the pregnancy rate reported in this study, embryos were cultured for 22 h before transfer and extended culture such as this has been shown to depress pregnancy rates. Second, variability in embryonic PA production was particularly high in embryos failing to generate pregnancies. Our laboratory has long suspected that a dying embryo may release PA in a burst because of membrane damage whereas a viable embryo would release PA in a controlled fashion. One solution to manage both the pregnancy rate and the uncontrolled PA release by dying embryos is to culture the embryos for a shorter period of time, e.g., 2-4 h. Although some time in culture is required to obtain measurable amounts of PA, subsequent embryo transfers could be performed within 4-5 h of collection in contrast to 22-24 h. This modification should have a twofold effect. First, pregnancy rate should increase because of the shorter time embryos are spent in culture and out of the uterus. Second, variability in PA production should also decrease because the shorter culture period would narrow the window of time where an embryo could die and spontaneously release PA. Therefore, the objective of the current work was to identify minimum culture

intervals where PA production by a single cow embryo could be measured.

Materials and Methods

Day 6 embryos were collected from donor cows maintained at the Oregon State University Beef Cattle Center. Cows were estrous-synchronized with two 25-mg injections of prostaglandin $F_{2\alpha}$ (Lutalyse) administered 12 days apart (Day 0 = first Lutalyse injection) and superovulated with twice daily injections of follicle stimulating hormone (Folltropin-V) at dosages of 50 mg on Days 10, 11, 12 and 13, respectively. Estrus detection started 24 h after the second injection of Lutalyse and cows were artificially inseminated with one straw of frozen bull semen at 0, 12 and 24 h after onset of estrus. At onset of estrus, all cows were injected with 100 µg of gonadotropin releasing hormone (GnRH; Fertagyl). Embryos were collected from cows 6 days after estrus by nonsurgically flushing the uterus with Dulbecco's phosphate buffered saline containing antibiotics and 0.2% heat-treated bovine serum. Embryos were recovered from the flushes, scored for developmental stage and quality and cultured in 15-µl microdrops of culture medium on 15 x 60 mm plastic tissue culture dishes in a humidified atmosphere of 5% CO_2 in air at 39°C. Medium was recovered from cultured embryos at 4, 12 and 18-h intervals and frozen at -20°C. Plasminogen activator activity in the culture medium was quantified using the caseinolytic agar gel assay described by Menino and Williams (1987).

Differences in embryonic PA production due to time in culture and quality scores were determined using analysis of variance (ANOVA). If significant effects were observed in the ANOVA, differences between means were evaluated by Fisher's least significant differences procedures. All analyses were performed using the NCSS statistical software program (Number Cruncher Statistical System; 2000, Jerry Hintze, Kaysville, UT).

Results

Significant PA production was measurable by a single embryo after only 4 h of culture providing embryos were at least a quality grade of good (Figure 1). Good embryos also produced significantly more PA than fair embryos during 4 and 12 h of culture. Fair embryos produced similar measurable PA after 4 and 12 h of culture but these levels were close to being barely detectable from the blank (data not shown) and may have represented embryos that were dying by 12 h. Fair embryos cultured for 18 h tended to produce less PA than good embryos cultured for only 4 or 12 h however this difference was not statistically significant. These data further corroborate results reported by Kaaekuahiwi and Menino (1989) who observed that embryos destined to shed their zonae pellucidae produced significantly more PA over 192 h of culture than embryos failing to reach this stage. These data are exciting because they illustrate the current assay is sensitive enough to measure PA production by a single cow embryo after as little as 4 h of culture.

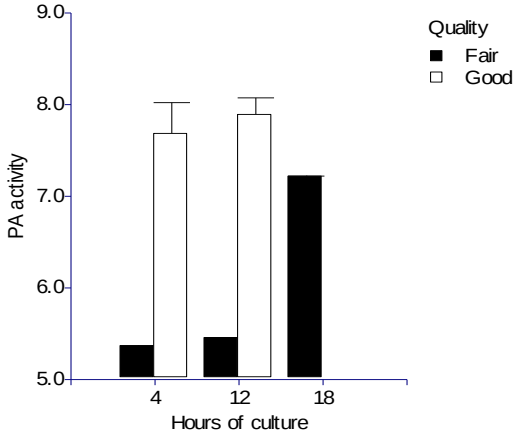

Figure 1. Mean plasminogen activator (PA) activities in medium recovered from cow embryos graded as good or fair quality and cultured for 4, 12 or 18 h. [a,b] Means without similar superscripts differ (P<0.05).

Conclusions

Results Measuring PA production by a single cow embryo has demonstrated some efficacy for selecting embryos most likely to generate pregnant recipients, however the extended 22-h duration of culture used in preliminary studies depressed overall conception rate. Current research has demonstrated that PA production can be measured by a single embryo cultured for as little as 4 h. Future work will be aimed at determining the shortest culture interval where PA production can be measured using the current assay. For this assay to have on farm applicability, PA production results should be available within an hour of collecting embryos. If the current assay is not sensitive enough with the shorter culture intervals, rapid and sensitive colorimetric and fluorometric assays are available that can be adapted to quantify embryonic PA production in a manner applicable to embryo transfer.

Acknowledgements

This research was generously supported by the Oregon Beef Council and the Oregon Agricultural Experiment Station.

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Oregon State University

BEEF098

Beef Cattle Sciences

Oregon Beef Council Report

Development of a diagnostic test for pregnancy detection in cattle [1]

Ruben Mendoza [2] and Alfred R. Menino, Jr [2]

Synopsis

Development and implementation of an assay for cattle pregnancy detection would benefit commercial cattle producers by eliminating the cost associated with maintaining non-pregnant cows.

Summary

The initial goal of this research was to adapt a kit that measures the plasma protein plasminogen activator inhibitor-2 (PAI-2) in humans for quantifying PAI-2 in cow plasma during pregnancy. In pregnant woman, plasma PAI-2 concentrations increase as gestation proceeds. Our laboratory has detected PAI-2 production by Day 14 cow embryos and this protein may serve as an indicator of pregnancy in cattle providing detectable quantities can be measured in the blood. Plasminogen activator inhibitor-2 concentrations during pregnancy were quantified in cow plasma and increased as gestation advanced. If plasma PAI-2 concentrations can be reliably measured during gestation in cattle, the long term goal is to develop a "dip-stick" type assay for pregnancy diagnosis similar to the "over-the-counter" products available for pregnancy detection in humans. However, difficulty was encountered in adapting the human kits for reliable and repeatable detection of PAI-2 in cow plasma. The kits use an antibody generated against human PAI-2 as the basis for their detection system. Therefore, current research has been aimed at screening antibodies from various vendors for recognition of bovine PAI-2 using Western blot analysis.

Introduction

Beef and dairy cattle producers are limited in the selection of approaches that can be used for pregnancy detection. Currently, there is no commercially available diagnostic kit for pregnancy detection in cattle that is comparable to the "over-the-counter" pregnancy tests available to humans. A diagnostic kit for progesterone is available that measures the relative amount of progesterone in the blood or milk. Progesterone is a hormone produced by the cow's ovary and remains high during pregnancy. However, this kit has limited application because approximately 75% of the time a non-pregnant cow will have progesterone levels indistinguishable from a pregnant cow. The BioPRYN (Pregnancy Ruminant Yes/No) procedure detects a pregnancy-specific protein in ruminant plasma known as pregnancy-specific protein B (PSPB) which is produced by the placenta. One limitation of BioPRYN is that the procedure requires the blood samples to be shipped to a laboratory for analysis. Although accuracy of detection of PSPB is fairly high and reports can be available in approximately a day, this assay protocol eliminates an "on the farm" test. Another limitation of BioPRYN is that it can be only used for early

1. This document is part of the Oregon State University – 2012 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.

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pregnancy detection (≈ Day 30 of pregnancy) in heifers. Cows that have calved will retain PSPB production until Day 90 of pregnancy thereby eliminating the availability of an early pregnancy test for the majority of the herd. Other techniques available to producers to detect pregnancy in cattle include examination of the reproductive tract via rectal palpation or ultrasonography. A veterinarian is usually called for the rectal palpation hence additional cost is incurred for the visit. Although detection of pregnancy by ultrasound approaches an acceptable percentage of correct diagnoses as early as 20 days after breeding, this procedure also requires a veterinary call and, this time, with specialized instrumentation.

The major advantage in accurate early pregnancy diagnosis is the culling of cows either artificially inseminated or exposed to bulls during the breeding season that have not become pregnant. Non-pregnant cows need to be eliminated from the herd as quickly as possible after the breeding season to avoid the expense of maintaining non-productive females. One estimate of the value involved in eliminating maintenance costs of non-pregnant cows is that for every 10% improvement in pregnancy rate there is a financial savings of 14% per pregnancy (Broadbent et al., 1991). This is of particular relevance to beef cattle producers who depend on returns from the calf crop. The ideal diagnostic kit would be one that producers could purchase cheaply and use on the farm without veterinary assistance.

For several years, our laboratory has been researching the protease plasminogen activator (PA) which is produced by cow embryos as early as Day 8 of pregnancy (Kaaekuahiwi and Menino, 1990; Singleton and Menino, 2005). Cow embryos also produce inhibitors of PA (PAI) that appear by Days 12-14 of pregnancy (Dyk and Menino, 1991). Interestingly, in humans, the placenta produces a specific pregnancy-associated PAI (PAI-2) that can be detected in the blood and increases as pregnancy progresses (Lecander and Astedt, 1986). Therefore, two questions emerged for the cow embryo PAI: 1) does the PAI produced by cow embryos appear in the blood during pregnancy, and if, yes, when, and 2) can the cow embryo PAI be distinguished from other PAIs normally found in the cow's blood? If the embryo PAI appears in the cow's blood before 21 days after breeding and it can be readily distinguished from other blood PAIs, then it would be feasible to develop a diagnostic kit for pregnancy detection in cattle. Western blotting was initially conducted to identify the type of PAI produced by cattle embryos. Culture medium and tissue extracts of Day 13-14 cattle embryos were probed with antisera to human PAI-1 and PAI-2. PAI-1 was observed in the culture medium and PAI-2 was detected in both culture medium and tissue extracts from Day 13-14 embryos.

Validating the identities of the PAI produced by the cow embryo allowed us to decide which PAI to pursue in developing a pregnancy test. Plasminogen activator inhibitor-1 is a normal constituent of plasma hence it would be least applicable of the two unless levels changed dramatically during pregnancy. Plasminogen activator inhibitor-1 concentrations change little in humans during pregnancy but no information of this nature is available in the cow. Plasminogen activator inhibitor-2 concentrations in cow plasma are near the detection limits of current assays however concentrations in humans increase during pregnancy. In a preliminary experiment using a kit from American Diagnostica, Inc. that measures PAI-2 in humans, we were able to detect PAI-2 in plasma from pregnant cows but not from non-pregnant cows. For these reasons, we proposed to pursue detection of PAI-2 in cow plasma for pregnancy diagnosis. Overall performance of the assay for quantifying cow PAI-2 was satisfactory however some troublesome between sample variability was encountered. During our attempts to modify certain aspects of the assay protocol to improve its precision, the vendor, American Diagnostica, Inc., discontinued production of the kit. Assays for PAI-2, unlike PAI-1, are scarce. Currently, only two vendors, both international, distribute kits. Our laboratory has evaluated kits designed for detection of PAI-2 in humans, rats, mice and rabbits from one vendor and these kits have not been successful in identifying the cow PAI-2.

In order to pursue this project, either an assay had to be created or a new assay identified and validated to measure cow PAI-2. The assay marketed by American Diagnostica, Inc. was an enzyme-linked immunosorbent assay (ELISA) that used two antibodies to detect human PAI-2. The antibodies in this assay also cross-reacted with cow PAI-2 which was why it could be measured in cow plasma. Our laboratory has experience in developing and conducting ELISAs hence two strategies were conducted simultaneously; develop direct and indirect ELISAs for measuring cow PAI-2. However, antibodies that recognize PAI-2 are only available for humans, rats, mice, guinea pigs and rabbits and a source for an antibody that specifically identifies cow PAI-2 has not yet been identified.

Therefore, our laboratory started on the path of searching for and testing antibodies that would recognize cow PAI-2 using Western blot analysis of embryos.

Materials and Methods

Embryos were collected from donor cows maintained at the Oregon State University Beef Cattle Center. Cows were estrous-synchronized with two 25-mg injections of prostaglandin $F_{2\alpha}$ (Lutalyse) administered 12 days apart (Day 0 = first Lutalyse injection) and superovulated with twice daily injections of follicle stimulating hormone (Folltropin-V) at dosages of 50 mg on Days 10, 11, 12 and 13, respectively. Estrus detection started 24 h after the second injection of Lutalyse and cows were artificially inseminated with one straw of frozen bull semen at 0, 12 and 24 h after onset of estrus. At onset of estrus, all cows were injected with 100 µg of gonadotropin releasing hormone (GnRH; Fertagyl). Embryos were collected from cows 14 days after estrus by nonsurgically flushing the uterus with Dulbecco's phosphate buffered saline containing antibiotics and 0.2% heat-treated bovine serum. Day 14 embryos were recovered from the flushes, scored for quality and either frozen immediately in liquid nitrogen or cultured in 100-µl microdrops of culture medium on 15 x 60 mm plastic tissue culture dishes for 48 h in a humidified atmosphere of 5% CO_2 in air at 39°C. Medium (conditioned medium) was recovered from cultured embryos at 24-h intervals and frozen at -20°C. At the end of the 48-h culture, embryos were recovered from the culture drops, scored for quality and frozen in liquid nitrogen.

For Western blot analysis, embryos, culture medium and human PAI-2 standards were combined with denaturing and reducing sample buffer and electrophoresed in 10% polyacrylamide gels. Gels were blotted unto nitrocellulose membranes (the Western blot) and blots were probed with either rabbit or goat antiserum prepared against human PAI-2.

Results

Our laboratory has evaluated 7 antibodies prepared against human PAI-2 from 3 vendors for cross-reactivity with the PAI-2 produced by Day 14 cow embryos. Of the 7 antibodies tested to date, only one has resulted in a positive signal against the cow embryonic PAI-2 (Figure 1). Using this particular antibody, two strong signals (arrows) were detected in embryo tissues and only a faint high molecular mass band was observed in medium recovered from cultured embryos. The high molecular mass band observed in both tissues and medium is probably a PA-PAI-2 complex as PAI-2 is known to rapidly complex with secreted PA. The low molecular mass band observed only in embryos is most likely free PAI-2 not in a complex with PA. Our laboratory will continue to screen PAI-2 antibodies from different sources using western blotting of Day 14 cow embryos to secure suitable cross-reacting antibodies that can be used in developing a reliable ELISA for detection of PAI-2 in cow plasma.

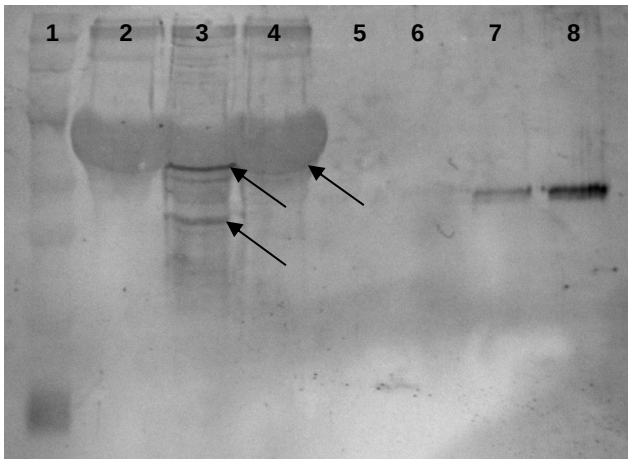

Figure 1. Western blot depicting PAI-2 standards and tissue and culture medium recovered from Day 14 cow embryos. Lanes contain 1) molecular mass markers, 2) culture medium without an embryo, 3) solubilized Day 14 cow embryo, 4) medium recovered from a Day 14 cow embryo after 24 h of culture, 5) 1 ng PAI-2, 6) 5 ng PAI-2, 7) 10 ng PAI-2 and 8) 50 ng PAI-2. Arrows point to positive signals for PAI-2 in cow embryo tissues and medium recovered from a cultured cow embryo.

Conclusions

Early experiments using a kit designed for measuring human plasma PAI-2 were successful in quantifying an increase in plasma PAI-2 concentrations as gestation advanced in cattle. Although this kit has been discontinued and a suitable replacement has not been found, work has continued to find a source of antibody that would detect the bovine embryonic PAI-2.

Acknowledgements

This research was generously supported by the Oregon Beef Council and the Oregon Agricultural Experiment Station.

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Oregon State University

BEEF099

Beef Cattle Sciences

Oregon Beef Council Report

Effects of varying levels of dietary glycerol on digestibility and fermentation of low, medium and high quality forages [1]

Chad J. Mueller [2], Hannah DelCurto [3], and Grace Deboodt [4]

Synopsis

The inclusion of up to 20% diet glycerol can improve both intake and diet digestibility of low, medium, and high quality forages fed to beef cattle.

Summary

Three 4×4 latin square studies were used to evaluate the effect of feeding 0, 5, 10, or 20% dietary glycerol with either a low (wheat straw), medium (grass hay), or high (alfalfa hay) quality forage to beef steers. Four ruminally-fistulated steers were used throughout the project, and were allowed to adapt to each diet for 10 days prior to collection. During the collection period intake, fecal output, and rumen liquid was monitored and sampled to determine digestibility and rumen fermentation changes. In-situ bags containing each fiber source was also included during the collection periods to determine fiber digestibility. The inclusion of glycerol improved intake, with 20% being greater than 0% when fed with grass and alfalfa hay. Total tract digestibility of both dry matter and organic matter increase with increasing glycerol inclusion for both wheat straw and grass hay, but had no impact on alfalfa digestibility. Regardless of forage source, acetate-to-propionate ratios decreased linearly with increasing glycerol inclusion level. The primary shift in rumen fermentation was from acetate to butyrate production, with minimal impacts on propionate production. Glycerol can be included in forage diets without negatively impacting intake or overall diet digestibility, but may reduce forage digestibility.

Introduction

The current expansion of the ethanol and bio-diesel industries around the U.S. will result in greater amounts of co-products available to various livestock industries. Currently the bio-diesel industry generates a protein meal (type depends crop source) and glycerol (or glycerin) as co-products. Typically the protein meal is sold to various livestock sectors as a protein supplement, and the glycerol is sold mainly to the cosmetics and pharmaceutical industries. Various personal sources have indicated that the current glycerol markets have adequate quantities for their manufacturing needs; therefore an increase in bio-diesel production will result in greater, and cheaper, quantities of glycerol for other entities. The combination of increased energy feedstuff prices (both grain and forage sources) and potential increases in glycerol production may result in operators attempting to

1. This document is part of the Oregon State University – 2012 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
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utilize glycerol to replace or supplement traditional dietary energy sources.

To date, most glycerol research has revolved around transitional or ketotic dairy cows and starch-based beef cattle diets. Little to no data exists to determine if or how glycerol can be used in low- to medium-quality forages such as those used in beef cow-calf operations. The current project was designed to evaluate the potential value of supplemental glycerol in beef cattle diets based on low, medium or high quality forages.

Materials and Methods

All procedures involving animals were approved by the Oregon State University Institute of Animal Care and Use Committee. Three feeding periods utilizing 4 ruminally-fistulated steers were used to evaluate consumption and overall digestibility of three different forages in conjunction with four different dietary inclusion levels of crude glycerol. Feeding periods were categorized based on forage source with steers being fed wheat straw (LOW) in period 1, alfalfa hay (HIGH) in period 2, and mixed grass hay (MED) in period 3. Within each period, dietary glycerol was included at 0, 5, 10, or 20% (DM basis) of the diet. During the feeding periods steers were maintained in individual pens located in the EOARC-Union metabolism barn.

The following procedures were common across all three periods. Prior to the start of a feeding period, steers were weighed and randomly allotted to dietary treatment.

Feeding

Steers received their respective diet (Table 1) for 10 d prior to the collection period. Steers were fed once per day (0800 h) with the exception of the LOW period in which steers were fed twice per day (0800 and 1630 h) due to bulkiness of the diet and limited feeder space. Individual ingredients were weighed and hand mixed on a daily basis just prior to feeding. Feed refusals (ORTS) were weighed and sampled prior to feeding the following day.

Collection period

Collection periods started on d 11 of feeding and continued through d 15. Prior to feeding on d 11, steers were fitted with fecal collection bags. Fecal bags were replaced with clean bags every 12 h through d 15. Once a 12 h collection was completed all fecal material was emptied into a pre-weighed container and re-weighed to determine 12 h fecal output. Fecal material was then hand mixed, subsampled (10% on a wet basis), sealed in an air-tight bag, and frozen until analysis. Fecal bags were then rinsed and allowed to dry prior to the start of the next 12 h collection period.

Prior to feeding on d 11, sixteen *In-situ* bags containing approximately 0.5 g of the parent fiber source was contained in a wide-mesh nylon bag and placed in the rumen. Four *In-situ* bags were removed at 4, 8, 12, and 24 hr to determine rate of DM and fiber digestibility. Once bags were removed they were rinsed in cold water until water was clear, stored in air-tight bags, and frozen until bags could be dried. This process was repeated on d 13 and 15 to account for variation in rumen environment. Rumen fluid samples were collected prior to feeding on d 11 for initial analysis of volatile fatty acids (VFAs) and rumen ammonia (NH_3). Once the initial rumen fluid samples were collected, the rumen was infused with 100 ml of CrEDTA. Rumen liquid samples were then collected at 4, 8, 12, and 24 h post-infusion. At time of rumen fluid collection, 100 ml of strained fluid was analyzed for pH and then subsampled, preserved in collection tubes, and frozen for later analysis of rumen volatile fatty acids (VFA), ammonia, and chromium (Cr) content.

Post-collection period

After the collection period was completed the steers were given access to a common outdoor pen for 24 to 48 h. Steers were then weighed, relocated to their designated individual pens, and assigned to their next diet. This process was repeated until all 4 steers received all 4 diets within the feeding period.

Feed, fecal, and *In-situ* bag samples were analyzed for dry matter (DM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) concentrations. All samples were dried at 60°C for 48 hr, except for fecal samples which were dried for 72 hr. All orts were dried at 60°C for 24 hr, then weighed to determine actual DM intake.

Statistical analysis

Data was analyzed as a repeated Latin square with Steer and Glycerol Inclusion as main effects and forage source as the individual Latin square. Steer was the experimental unit.

Results

Wheat straw

No differences ($P > 0.10$) were detected for DMI as a percentage of BW (table 2) across glycerol inclusion levels. Values for apparent total tract digestibility of dry matter (ATTDMD), neutral detergent fiber (ATTNDFD), and acid detergent fiber (ATTADFD) are presented in table 2. There was a linear increase in ATTDMD across glycerol inclusion levels, resulting in a 23.5% increase in overall ATTDMD for 20% glycerol compared to 0%. No differences ($P > 0.10$) were detected for ATTNDFD or ATTADFD across glycerol inclusion levels. The combination of the ATTDMD and fiber digestibility data would suggest that the changes in DM digestibility was solely the result of digesting additional non-fiber nutrients, with minimal impact on fiber digestibility. In-situ digestibility (table 2) of DM was lowest for 20% glycerol, with 10% glycerol intermediate to 0, 5 and 20%. There were no differences in in-situ NDF digestibility across glycerol levels, but in-situ ADF digestibility was greater for 0% compared to 20% glycerol. The in-situ data indicates that glycerol reduce forage digestibility, with the greatest impact on inclusions greater than 10%. Inclusion of glycerol had minimal effect on rumen pH, but resulted in a linear decrease ($P < 0.05$) in acetate-to-propionate ratio. The primary driver was a shift from acetate production to butyric acid (table 2). Butyrate concentrations increased as glycerol inclusion increased, while there was minimal change in propionate production. Rumen NH_3-N was similar ($P > 0.10$) between 0, 5, and 20% glycerol; and 10% glycerol tended to be lower than 0, but not different than either 5 or 20% glycerol.

Grass hay

Dry matter intake as a percentage of body weight, ATTDMD, and ATTOMD increased with glycerol inclusion rate (table 3). Digestibility of diet NDF was greatest in 5% glycerol diets. In-situ DM and NDF forage digestibility was lowest for 20% glycerol compared to any other inclusion rate. Acetate-to-propionate ratio decreased linearly ($P < 0.05$) as glycerol inclusion increased (table 3), which was driven primarily by a linear reduction in acetic acid production. Similar to the LOW diet, propionic acid remained constant across inclusion rates, whereas butyric acid increased linearly ($P < 0.05$) with increasing glycerol level. Rumen NH^3-N was lowest for 5 and 10% glycerol compared to 0 and 20% glycerol.

Alfalfa hay

Dry matter intake as a percentage of body weight was highest for 20% compared to 0%, with 5 and 10% glycerol DMI intermediate (table 4). Similar to both wheat straw and grass hay, as inclusion rate of glycerol increased so did the ATTDMD and ATTOMD of the diet (table 4). There were no differences in ATTNDFD, and ATTADFD was higher for 5% compared to 20% glycerol. There were no differences observed for in-situ DM, NDF, or ADF digestibility across glycerol inclusion rates (table 4). Inclusion of glycerol did not change rumen pH, but linearly decreased ($P < 0.05$) acetate-to-propionate ratio. Again the primary change in fermentation was from acetic acid to butyric acid production (table 4); with minimal effect on propionic acid production. Rumen NH_3-N decreased as glycerol inclusion increased, with 0% glycerol having the greatest concentration of NH_3-N.

Conclusions

Inclusion of glycerol in either LOW, MED, or HIGH forage diets resulted in improved diet digestibility, but typically reduced forage digestibility. Intake was also improved as glycerol inclusion rate increased, therefore glycerol can be used to improve the palatability of lower quality forages. From a rumen fermentation standpoint, the primary change in was a reduction in acetate production, and a linear increase in butyric acid production as glycerol inclusion was increased. Though a cost analysis was not performed, the diet digestibility and intake data would suggest that including glycerol up to 20% of the diet can positively impact the use of low and medium quality forages feed to beef cattle.

Acknowledgements

The authors would like to thank the Oregon Beef Council for their financial support, and Dr. Tim Bodine and PerforMix for donating the crude glycerol to the project.

Table 1. Diet formulations of wheat straw (LOW), grass hay (MED), and alfalfa hay (HIGH) supplemented with various levels of glycerol.

Item	Glycerol inclusion, % DM			
	0%	5%	10%	20%
			LOW[a]	
Wheat straw[b], % DM	84.92	79.97	74.97	64.80
Glycerol, % DM	0.00	4.48	9.01	18.20
Soybean meal, % DM	15.08	15.55	16.02	17.00
Nutrient analysis[c]				
Dry matter, %	90.16	89.95	89.74	89.32
Crude protein, %	9.77	9.87	9.97	10.18
NDF[d], %	70.38	66.52	62.63	54.71
ADF[e], %	41.94	39.57	37.18	32.31
Ash, %	8.76	8.37	7.97	7.17
			MED[a]	
Grass hay[b], % DM	95.74	90.16	84.50	72.98
Glycerol, % DM	0.00	4.42	8.89	18.01
Soybean meal, % DM	4.26	5.42	6.61	9.01
Nutrient analysis[c]				
Dry matter, %	91.54	91.27	90.99	90.46
Crude protein, %	9.76	9.88	10.02	10.28
NDF[d], %	59.14	55.99	52.80	46.29
ADF[e], %	31.39	29.64	27.87	24.26
Ash, %	13.54	12.87	12.20	10.83
			HIGH[a]	
Alfalfa hay[b], % DM	100.00	95.72	91.29	82.33
Glycerol, % DM	0.00	4.28	8.71	17.67
Nutrient analysis[c]				
Dry matter, %	93.51	93.14	92.77	92.02
Crude protein, %	16.74	16.02	15.28	13.78
NDF[d], %	50.44	48.29	46.05	41.53
ADF[e], %	36.74	35.16	33.54	30.24
Ash, %	10.26	9.82	9.37	8.45

[a]LOW=low quality forage diet (wheat straw), MED = medium quality forage diet (mixed grass hay), and HIGH = high quality forage diet (alfalfa hay). All values were calculated based on actual feed amounts delivered and dry matter.
[b]Forages were coarsely chopped (approximately 4 to 6 in. particle length).
[c]Based on laboratory analyses. Values are reported on DM basis.
[d]Neutral detergent fiber.
[e]Acid detergent fiber.

Table 2. Digestibility and rumen fermentation characteristics of wheat straw (LOW) mixed with various inclusion rates of glycerol.

Item	Glycerol inclusion, % dietary DM				SEM
	0%	5%	10%	20%	
DMI, % BW[1]	1.80	1.83	1.87	2.07	0.114
Apparent total tract digestibility					
DM, %	50.96^{e}	52.34^{e,g}	56.63^{a,g,h}	61.84^{b,f,h}	1.088
OM, %	55.12^{a,e}	56.70^{a,e,f}	60.28^{b,c,f,g}	64.13^{d,g}	0.847
NDF, %	51.42^{e}	49.23^{e}	51.68^{e}	56.15^{f}	0.861
ADF, %	44.95	46.40	50.25	55.01	2.619
In-situ rumen digestibility[2]					
DM, %	10.13 ± 0.39^{e}	10.45 ± 0.94^{a,e}	9.63 ± 1.12^{b,e}	7.47 ± 0.45^{f}	
NDF, %	20.32 ± 4.03	19.95 ± 5.39	19.00 ± 5.64	16.15 ± 1.32	
ADF, %	22.24 ± 1.79^{e}	20.78 ± 3.04^{a}	20.00 ± 3.92^{a}	16.84 ± 1.33^{b,f}	
Rumen fermentation[2]					
Rumen pH	6.81 ± 0.07^{a}	6.80 ± 0.06^{a}	6.82 ± 0.09^{a}	6.73 ± 0.06^{b}	
Acetate:propionate ratio	1.97 ± 0.05^{e}	1.71 ± 0.04^{f}	1.50 ± 0.10^{g}	1.26 ± 0.10^{h}	
Acetic acid, mol/ 100 mol	50.14 ± 0.49^{e}	43.90 ± 0.60^{f}	40.02 ± 0.82^{g}	29.05 ± 0.91^{h}	
Propionic acid, mol/100 mol	25.51 ± 0.56	25.92 ± 0.71	27.18 ± 1.77^{a}	23.86 ± 2.31^{b}	
Butyric acid, mol/100 mol	15.93 ± 0.73^{a,e}	21.21 ± 1.40^{b,e,f}	24.43 ± 2.10^{f}	36.10 ± 2.73^{g}	
Isobutyric acid, mol/100 mol	2.25 ± 0.03	2.30 ± 0.14	2.18 ± 0.12	2.28 ± 0.13	
Valeric acid, mol/100 mol	2.08 ± 0.14^{e}	2.30 ± 0.13^{e}	2.24 ± 0.12^{e}	3.79 ± 0.23^{f}	
Isovaleric acid, mol/100 mol	4.08 ± 0.08^{e}	4.37 ± 0.17^{a,e}	3.96 ± 0.55^{e}	4.92 ± 0.20^{b,f}	
NH_3-N, mg/dl	2.75 ± 0.60^{a}	2.35 ± 0.22	1.75 ± 0.39^{b}	2.36 ± 0.28	

[1]Dry matter intake as a percentage of shrunk body weight (lb). Body weights were adjusted 5% for gut fill.
[2]Mean ± standard error.
[a,b,c,d]Means within a row with different superscripts differ ($P < 0.10$).
[e,f,g,h]Means within a row with different superscripts differ ($P < 0.05$).

Table 3. Digestibility and rumen fermentation characteristics of grass hay (MED) mixed with various inclusion rates of glycerol.

Item	Glycerol inclusion, % dietary DM				SEM
	0%	5%	10%	20%	
DMI, % BW[1]	1.97[e]	2.02[e]	2.04	2.14[f]	0.022
Apparent total tract digestibility					
DM, %	58.74[e]	62.32	61.45	65.87[f]	0.902
OM, %	64.74[e]	67.84	67.02[a]	70.89[b,f]	0.773
NDF, %	54.89	56.03[e]	50.80[f]	53.42	0.851
ADF, %	41.54	49.25	42.55	41.43	2.104
In-situ rumen digestibility[2]					
DM, %	30.05 ± 0.85[e]	29.60 ± 0.58[e]	29.77 ± 0.85[e]	27.94 ± 1.16[f]	
NDF, %	39.83 ± 1.27[e]	39.33 ± 0.96[e]	39.92 ± 1.76[e]	36.17 ± 2.00[f]	
ADF, %	37.32 ± 1.13	36.03 ± 2.3	36.32 ± 0.43	35.35 ± 2.22	
Rumen fermentation[2]					
Rumen pH	6.59 ± 0.09[a,e]	6.67 ± 0.10[b]	6.67 ± 0.04[b,f]	6.60 ± 0.09[a]	
Acetate:propionate ratio	2.13 ± 0.04[e]	1.87 ± 0.07[f]	1.69 ± 0.05[g]	1.29 ± 0.04[h]	
Acetic acid, mol/ 100 mol	50.71 ± 0.36[e]	42.65 ± 0.99[f]	37.91 ± 0.44[g]	31.19 ± 0.42[h]	
Propionic acid, mol/100 mol	23.85 ± 0.30[a,e]	22.79 ± 0.43[b,e,f]	22.29 ± 0.43[f]	24.22 ± 0.74[e,g]	
Butyric acid, mol/100 mol	19.44 ± 0.29[e]	27.20 ± 0.66[f]	31.87 ± 0.10[g]	35.00 ± 0.63[h]	
Isobutyric acid, mol/100 mol	1.45 ± 0.02[e]	1.66 ± 0.03[f]	1.71 ± 0.08[f]	1.68 ± 0.10[f]	
Valeric acid, mol/100 mol	2.18 ± 0.07[e]	3.04 ± 0.09[f]	3.44 ± 0.24[f]	4.88 ± 0.22[g]	
Isovaleric acid, mol/100 mol	2.36 ± 0.11[e]	2.66 ± 0.19[f]	2.77 ± 0.13[a,f,g]	3.02 ± 0.13[b,g]	
NH_3-N, mg/dl	1.43 ± 0.16[e]	1.18 ± 0.19[f]	1.20 ± 0.31[f]	1.46 ± 0.18[e]	

[1]Dry matter intake as a percentage of shrunk body weight (lb). Body weights were adjusted 5% for gut fill.
[2]Mean ± standard error.
[a,b,c,d]Means within a row with different superscripts differ ($P < 0.10$).
[e,f,g,h]Means within a row with different superscripts differ ($P < 0.05$).

Table 4. Digestibility and rumen fermentation characteristics of alfalfa hay (HIGH) mixed with various inclusion rates of glycerol.

	Glycerol inclusion, % dietary DM				
Item	0%	5%	10%	20%	SEM
DMI, % BW[1]	2.62[e]	2.74	2.69	2.82[f]	0.037
Apparent total tract digestibility					
DM, %	64.17[a,e]	66.47[a,b,c]	66.93[b]	69.26[d,f]	0.555
OM, %	64.97[a,e]	67.33[a,b,c]	67.96[b]	70.46[d,f]	0.608
NDF, %	51.33	53.83	51.61	49.65	1.327
ADF, %	50.94	54.70[a]	51.94	49.40[b]	1.235
In-situ rumen digestibility[2]					
DM, %	48.23 ± 0.64	48.64 ± 0.49	47.43 ± 1.15	47.71 ± 0.59	
NDF, %	56.97 ± 0.74	57.77 ± 1.04	55.70 ± 2.42	56.71 ± 1.28	
ADF, %	55.45 ± 1.20	56.82 ± 0.95	55.12 ± 1.25	55.59 ± 1.07	
Rumen fermentation[2]					
Rumen pH	6.67 ± 0.04	6.71 ± 0.05	6.70 ± 0.08	6.72 ± 0.13	
Acetate:propionate ratio	2.15 ± 0.05[e]	1.90 ± 0.02[f]	1.70 ± 0.10[g]	1.46 ± 0.02[h]	
Acetic acid, mol/ 100 mol	50.15 ± 0.67[e]	43.68 ± 0.65[f]	38.63 ± 1.56[g]	30.84 ± 1.66[h]	
Propionic acid, mol/100 mol	23.62 ± 0.30[a]	23.50 ± 0.15[a]	23.13 ± 0.47[a,b]	21.36 ± 1.14[b]	
Butyric acid, mol/100 mol	15.53 ± 0.19[a,e]	21.79 ± 0.47[b,e,f]	26.82 ± 0.95[c,f]	36.15 ± 3.04[g]	
Isobutyric acid, mol/100 mol	2.46 ± 0.08[e]	2.38 ± 0.04[e]	2.36 ± 0.16[a]	2.17 ± 0.13[b,f]	
Valeric acid, mol/100 mol	4.05 ± 0.14[e]	4.64 ± 0.24[a,f]	5.23 ± 0.36[b,f]	5.72 ± 0.13[c,g]	
Isovaleric acid, mol/100 mol	4.18 ± 0.20	4.01 ± 0.07	3.83 ± 0.09	3.77 ± 0.31	
NH_3-N, mg/dl	5.36 ± 0.40[a,e]	4.71 ± 0.37[b,f]	4.18 ± 0.32[f]	2.36 ± 0.38[g]	

[1]Dry matter intake as a percentage of shrunk body weight (lb). Body weights were adjusted 5% for gut fill.
[2]Mean ± standard error.
[a,b,c,d]Means within a row with different superscripts differ ($P < 0.10$).
[e,f,g,h]Means within a row with different superscripts differ ($P < 0.05$).

Beef Cattle Sciences

Oregon Beef Council Report

Progress Reports – Animal Sciences [1]

A pilot study to evaluate in the association of metabolic disorders in early lactation and the incidence of anoestrus in dairy cows

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Project Objectives: To objectively measure the association between early metabolic disease and the incidence of anoestrus in dairy cattle.

Project Start Date*:* August 2010
Project Completion Date: August 2011

Project Status: A total of 90 cows have been enrolled in the study and all data have been recorded. Statistical analyses are to be performed by the end of the year. Preliminary results have been presented at various state, national and international venues including the World Buiatrics Conference in Chile, and two invited presentations in Italy and Israel.

During the analysis of the data collected for this study, it became apparent that there is a lack of appropriate standards for the Jersey breed. All laboratory standards have been developed using Holstein cows. This finding in itself will forever change the way cattle research is done. Appropriate standards will need to be established for different breeds so that underlying physiology can be correctly evaluated. It makes sense that, if different breeds of cattle have different production levels or specific production characteristics (such as high protein in milk for dairy cows or higher marbling in beef cattle), they may have different underlying physiological explanations for those different traits. We need to explore what they are, so that they can be appropriately evaluated.

The significance of this finding for the current project lays in the fact that the data cannot be appropriately analyzed until Jersey standards have been developed. The most positive part though, is that we have received funding to establish these standards, and the project is underway. We expect to have the standards finalized by the end of the year, and then we will be able to correctly analyze and interpret the data from the study funded by the Oregon Beef Council. We truly appreciate the support of the council on this project. We envision a bright future for this project and those deriving from it.

1. This document is part of the Oregon State University – 2012 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.

Does Oral Administration of Selenium to Calves at Birth Increase Passive Absorption of Immunoglobulin G?

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Project Objectives: 1) To determine if oral drenching of dairy calves with selenium within hours of birth enhances passive transfer of IgG. 2) To determine if supranutritional selenium supplementation of cows enhances passive transfer of IgG in their calves.

Project Start Date*:* August 2011
Expected Project Completion Date: August 2013

Project Status: This project is another step in the process of evaluating the benefits of supranutritional levels of selenium in the health of beef cattle. The first study, funded by the Oregon Beef Council was completed in November of 2009. The title was Selenium Supplementation and Retention in Beef Cattle. Results showed higher concentrations of whole-blood selenium and a longer retention period after supplementation in cows grazing selenium-fertilized pastures compared to cows fed trace mineral mixture with selenium.

In the current project, a group of 32 dairy-production cows at the Boardman dairy were selected and fed an organic source of Se as selenomethionine-yeast added to a small amount of dry ration once weekly Cows were individually locked up and fed this Se treatment once weekly for a total of 8 weeks prior to calving. As these cows calved, their first colostrum was saved and frozen for later preparation. The calves from these cows were randomly assigned to treatment groups as described in the proposal protocol.

A second group of 32 dairy-production cows were selected to receive the usual dose of inorganic Se as part of their diet (3 mg/d Se is considered equivalent to 0.3 ppm Se in the diet; standard dose and FDA regulation). Their first colostrum was also saved and frozen for later preparation. The calves from these 32 cows were randomly assigned to treatment groups as described in the proposal protocol, which was essentially a cross-over study design.

Calves from the two groups of cows were fed two liters of colostrum within 2 hours postparturition and then 2 liters of colostrum at 12 hours postparturition. Depending upon the treatment group, calves received either a dose of sodium selenite or no selenium in the colostrum. Calves were subsequently kept in individual calf hutches.

Whole blood samples were collected from all cows at parturition to measure selenium concentrations. Whole blood and serum were collected from calves at birth and 48 hours to measure whole blood and serum selenium concentrations. Serum was collected at 48 hours and 14 days to determine IgG concentrations. Whole blood was collected at 30 days in all calves to measure WB-Se concentrations.

All blood samples were submitted for analysis of selenium concentrations to Michigan State University. IgG concentrations and titers are being assessed in Dr. Hall's lab using an indirect ELISA procedure. This process is currently underway. Once these studies are complete, then an analysis of the entire project will be completed.

Western Juniper - Induced Abortions in Beef Cattle

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Project Objectives: The goal of this study is to determine high, medium and low risk areas for potential juniper induced abortions in cattle and provide management recommendations to OSU extension, and Oregon cattle producers to reduce cattle losses. The objectives of this study are three fold:
1- Determine the extent of the potential variation in the concentration of abortifacient compounds in western juniper trees across cattle grazing regions of Oregon.
2- Determine if there are seasonal, geographical, or other factors which alter the concentration of abortifacient compounds in western juniper trees in Oregon.
3- Further assess the potential of western juniper trees to induce late term abortions in beef cattle by dosing more animals with plant material.
Duration of study: Objective 1 – two months to collect, prepare, chemically analyze, and catalog the samples.
Objective 2 – one year to purchase cows, time breed them, maintain them for six months in dry lot until 250 days gestation at which time cows will be dosed via stomach tube for 10 days (gestation days 250-259).
Objective 3 – two years to collect samples and then prepare and chemically analyze samples.

Project Start Date*:* Not informed
Expected Project Completion Date: Not informed

Project Status: Objective 1 has been accomplished with the final chemical analysis results having been summarized and interpreted, with results being submitted in manuscript form and accepted for publication in Rangelands. The results of objective 2 were that two out of the six pregnant heifers that were fed Western juniper bark prematurely aborted their calves. That is 33% of exposed cows aborting their calves, which would be beyond the point of recovery for most beef cattle producers to recuperate from and would be economically devastating to most any operation. We are also going to repeat the feeding trial in conjunction with another research trial we are conducting and are aiming for more pregnant cattle to be exposed. This second feeding trial will take place during the calving season of 2013. Objective 3 has been initiated with most of the first years of geographical juniper analysis having been collected. Once we finish the second year of tissue collection we will analyze the samples and create a geographical and seasonal report on Labdane acid concentration of Juniper in Eastern Oregon.The results of these studies will then be published in appropriate publications with the beef cattle producers across the state being better informed and educated regarding the potential of Western juniper to cause premature births, (abortions) in beef cattle.

The effect of western juniper (*Juniperus occidentalis*) on the estrous cycle of beef cattle

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Project Objectives: The objective of this research proposal is to determine if consumption of western juniper alters the estrous cycle of beef cows and subsequently extends the calving interval or reduces conception rates during the summer grazing season.

Duration of study: Two complete years; year one to collect the samples and purchase the cattle, then year two to grind the samples, feed the samples and monitor blood samples for impact on pregnancy hormones of the heifers during the breeding season. The pen study portion of this proposal will be completed in approximately a six month period. Bark will be collected in a one week period approximately two months before the beginning of the study. The cattle will be monitored for two months before feeding and two months after feeding. The data analysis will take approximately one month

Project Status: Three thousand pounds of Western juniper bark has been collected and will be tested for individual Labdane Acid compounds prior to the feeding and estrus research we will be conducting this coming spring. We will be purchasing the research heifers this winter in preparation for the spring of 2013 trial. Once the trial is complete we will analyze the data, write reports and submit to appropriate venues for consideration for publication.

Yeast Culture Supplementation May Improve Reproductive Performance in Cows

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Project Objectives: Evaluate the effect of 0, 56, or 112 g/d of yeast culture fed from 4 weeks pre-calving to 4 weeks post calving on reproductive performance of cows and serum concentrations of vitamin E, progesterone, and cholesterol.

Project Start Date*:* October 2012
Expected Project Completion Date: October 2014

Project Status: Multiparous Holstein cows (32 cows per group; 96 cows total) were fed individually in addition to their regular diet 0, 56, or 112 g/d of yeast culture (Diamond V XP®, Diamond V, Cedar Rapids, IA) from 4 weeks before calving to 4 weeks after calving. The animals were maintained at a commercial dairy and the study was approved by the Oregon State University Animal Care and Use Committee. Blood samples were taken 28, 21, 14, 7, 3, and 1 day before the anticipated calving date, at calving, and 1, 3, 7, 14, 21, 28, 35, 42, and 49 days after calving. Milk samples were taken twice a week. Reproductive events were monitored until the cow calved or until the cow left the farm, whichever came first. We started to quantify serum concentrations of vitamin E, progesterone, and cholesterol in blood samples. As soon as all serum samples are analyzed and statistically summarized, the results will be published in the next edition of the Oregon Beef Council Report, and presented at extension and scientific meetings. The results will be published into extension materials and scientific literature.

The Economics of Grassed-Based Dairying in Oregon

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Project Objectives: The objective of this project is to help these dairymen design a system to successfully document pasture growth, feed quality, utilization and milk production. In January 2012, I started organizing this project by defining all the grazing pastures at each operation. Data collection will end mid-November 2012 when our grazing season is complete.

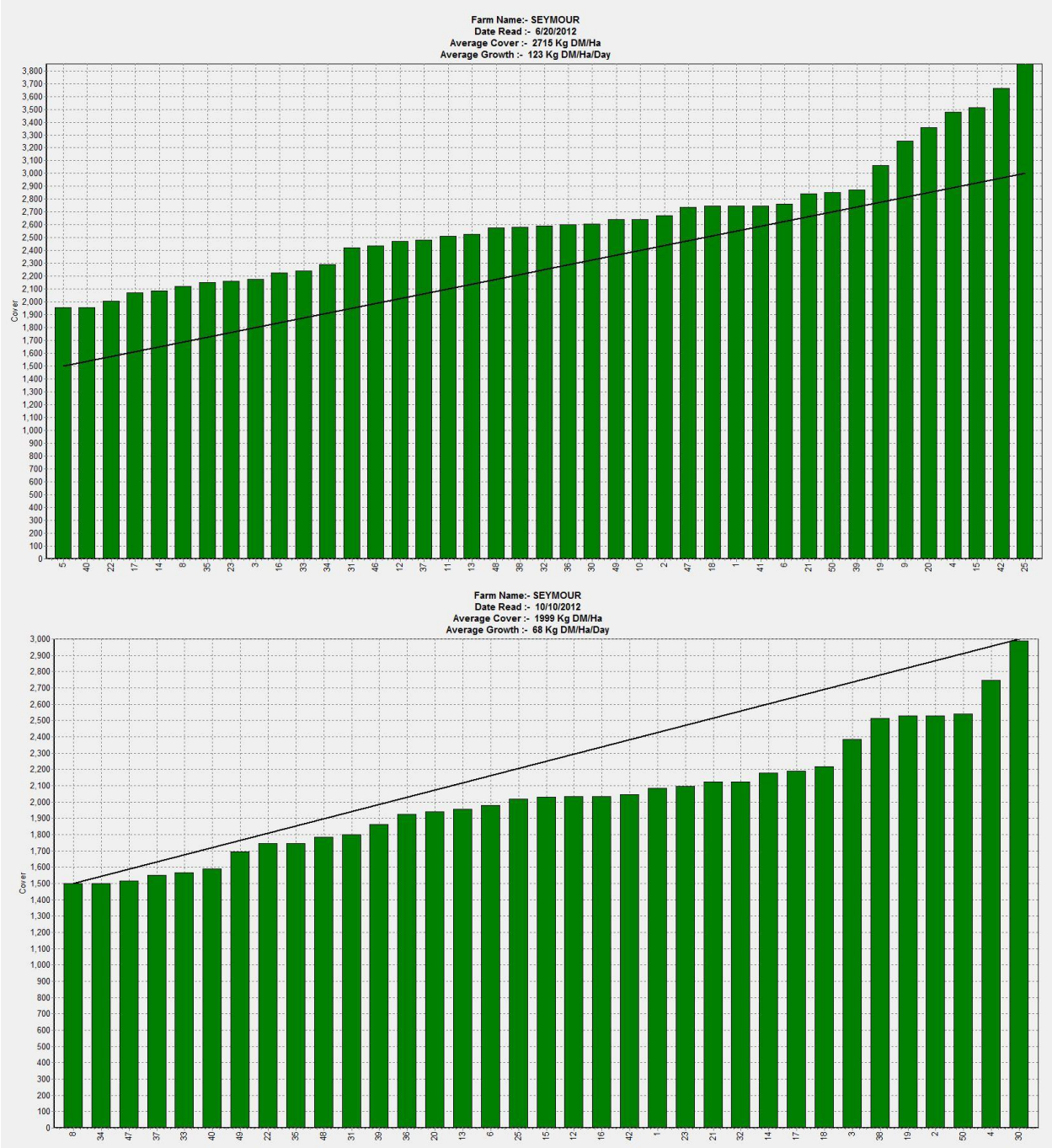
Project Start Date*:* Not informed
Expected Project Completion Date: Not informed

Project Status: One of the cooperating dairies is in Cloverdale and has 43 pastures that average around 6 acres each for milk cow grazing. Fields were measured with a GPS unit and mapped. The second dairy included in this project has 22 pastures that average 5 acres each for their milk cow rotations. Management software was ordered and pasture cover measuring equipment was purchased to get ready for data collection to start. Additionally, a paper record keeping system was develop with the participating dairyman to be used on the dairy to record daily pastures grazed, residual pasture height and supplemental feed fed in the barn. The goal was to be able to account for daily pasture consumption, supplemental feed fed, and milk produced. Both these dairies grazed intensively often moving to a new paddock with 12-24 hours.

Depending on the year, the first turn out often occurs between Feb 15 and March 15. This year cows were turned out March 1 and this is when our intensive data collection began. Forage cover measurements were measured weekly on all 65 pastures in this project and entered into management software. Paddock grazing and residual heights were also included in the electronic recordkeeping. Weekly grazing wedges were generated on farm and left for operators to use to make management decisions. Initially the data was considered interesting but within a few weeks producers were anxious to see the new wedges and use that inventory information to make better decisions.

Grazing wedges were printed weekly (>7 months now) after inventorying standing forage and entering the dry matter records into grazing management software. These grazing wedges show graphically all the pastures on the farm and the total standing dry matter that week. They also show growth rate for the week expressed as pounds of dry matter per acre (kg/hec). Below are examples of two wedges. The black line running across the graph is a theoretical “ideal” if growth where constant. Typically we graze pastures down to a residual of 1500 lbs. /acre of dry matter and theoretically harvest or graze around 3000. In the first graph the total farm grass inventory is significantly larger than the idea which indicates our growth rate is exceeding our ability to keep up. In this case the first seven paddocks with the most grass were harvested as silage to stay ahead of the surplus.

When grass growth slows you can see grass inventories look like the second grazing wedge. In this case it appears we will have a grass shortage in the next 7-10 days with the pasture possibly changing up later in the rotation. Offering additional supplement is often what happens with this condition until animal numbers can be more closely matched with pasture feed inventories.



Forage Quality Results: The majority of the weekly grass samples have been harvested and analyzed for the first year. Listed below is the average quality of each of the two farms for the 28 samples taken so far.

	DM	protein	Deg. P	NDF	NDFD	RFV	WSC	NEl	ME
# 1	15.1	23.3	73.8	46.5	80.8	140.3	18.7	.68	1.20
# 2	15.7	22.5	71.7	48.3	77	131.4	20.6	.65	1.17

	CA	P	Mg	K	Na	Fe	Zn	Cu	Mn
#1	.42%	.40%	.18%	3.6%	.09%	216	40	9.5	78.8
#2	.45%	.39%	1.9%	3.6%	.06%	239	27	9.6	53

Influence of Supplement Composition of Utilization of Low-Quality Cool-Season Forage

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Project Objectives: We hypothesize that, compared with warm-season forages, the high proportion of ruminal degradable protein and greater fiber digestibility normally observed with cool-season forages will result in high energy supplements with a low level of CP (< 15%) being superior to higher CP (> 15%) supplements. This would alter our currently recommended supplementation strategies for ruminants consuming low-quality cool-season forages and may reduce costs associated with supplementation while improving animal performance; thereby increasing economic returns of Oregon's beef producers.

Project Start Date*:* September 1, 2012
Expected Project Completion Date: November 1, 2013

Project Status: This project entails three experiments. The first experiment, a heifer supplementation study has been completed and we are currently in the process of conducting laboratory analyses. The second study, nutrient digestion study, will begin in late-October of 2012 and conclude in late-February 2013. We anticipate laboratory analyses to be completed by August of 2013. The third study, a cow performance study, will begin in mid-December and conclude in mid-April. We anticipate all data to be analyzed by August of 2013. We should have a couple of extension publications and at least two peer-reviewed, professional journal articles published as a result of this project.

Comparison of Ivomec Plus® and a Generic Anthelmintic on Parasite Control and Performance of Beef Cattle

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Project Objectives: Compare performance, production efficiency, and health responses of beef cattle exposed to parasitic infections and treated with Ivomec® Plus or Noromectin® Plus. At the completion of this research, we expect to determine if beef producers can effectively use the less expensive generic product as an anthelmintic treatment and expect the same cattle health and performance responses compared with the traditional brand. This proposal is significant to the Oregon beef industry by generating information that can reduce production costs in beef operations, further encourage producers to adopt strategies to control cattle parasites, and ultimately decrease the incidence of parasitic infestations in Oregon beef herds.

Project Start Date*:* September 1, 2013
Expected Project Completion Date: December 1, 2013

Project Status: This project will consist of 2 experiments that will start when the EOARC calves are weaned in 2013. The specific objective of experiment 1 will be to compare the efficacy of Ivomec® Plus and Noromectin® Plus in reducing or eliminating internal parasitic infection in weaned calves. The specific objective of experiment 2 will be to compare the efficacy of Ivomec® Plus and Noromectin® Plus in preventing the infestation of internal parasites in weaned calves. Upon completion of this study, we expect to determine if the generic product (Noromectin® Plus) can be used by beef producers as a less expensive but effective alternative to the brand product (Ivomec® Plus) in eliminating or preventing internal parasitic infections in beef cattle. This proposal is significant to Oregon beef producers by providing options to decrease costs of production, demonstrating the importance and economic benefits of anthelmintic treatments in beef operations, and ultimately encouraging producers to adopt strategies that control and decrease the incidence of parasitic infestations in Oregon beef herds.

Oregon State University

BEEF093

Beef Cattle Sciences

Oregon Beef Council Report

Potential benefits of sagebrush (*Artemisia tridentata*) consumption by cattle [1]

J. A. Perez-Amaro [2], R. Mata-Gonzalez [3] and T. DelCurto [4]

Synopsis

We documented an increase in steer body weight resulting from the inclusion of small doses of sagebrush (*Artemisia tridentata*) in hay diets. Sagebrush inclusion in the diets, up to 5%-6%, seems to be beneficial for weight gain. However, higher rates of sagebrush in the diet might have detrimental effects.

Summary

The objective of this study was to determine the impact of small proportions of sagebrush in the diet of steers. A feeding trial experiment was conducted on cannulated crossbred steers. Dietary treatments consisted of increasing proportions of sagebrush in a basic grass-hay diet of poor nutritional quality. The sagebrush proportions were 0%, 0.5%, 1%, 3%, and 9% in dry weight basis. Results indicated that sagebrush additions can benefit cattle. The weight gains were successfully modeled by a quadratic regression in which the maximum positive response seemed to correspond to a dose of 5%-6% of sagebrush in the diet. Voluntary hay consumption was not affected by the sagebrush inclusions. We suggest that small doses of sagebrush in cattle diets might be beneficial by improving conversion efficiency. Further research is needed to elucidate the physiological reasons underlying this response.

Introduction

Sagebrush occurs on millions of hectares and is a dominant component of North American rangelands (West et al., 1978). Because of its abundance, sagebrush can be important forage for wildlife and livestock, but the presence of secondary metabolites in the foliage limits its nutritional value (McInnis and Vavra, 1987; Villalba et al., 2006). Previous research has shown that sagebrush produces plant secondary metabolites, such as terpenes, which serve the purpose of deterring its consumption by herbivores (Villalba et al. 2006). Many herbivores limit the intake of otherwise nutritious plants such as sagebrush because of the toxicity posed by plant secondary metabolites. Plant secondary metabolites might cause post-ingestive consequences such as weight loss and even death. However, it has lately been established that plant secondary metabolites in small amounts can promote good animal health due to their anti-parasitic, anti-bacterial, and anti-fungal properties (Villalba and Provenza 2007). Thus, the difference between the toxic and medicinal effects of sagebrush can be very small, merely a matter of dosage.

It has been determined that sagebrush is not a substantial component of cattle diets, but it is clear

1. This document is part of the Oregon State University – 2012 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.
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that some consumption of sagebrush usually occurs in rangelands (McInnies and Vavra, 1987). It is possible that rangeland herbivores such as cattle limit their intake of sagebrush, while not totally avoiding it. We hypothesize that appropriately small doses of sagebrush can have beneficial effects on cattle.

Materials and Methods

A feeding trial experiment was conducted at the facilities of the Animal Sciences Department of OSU. Treatment of the experimental steers was in accordance with regulations of the Oregon State University Animal Care and Use Committee. The purpose of the experiment was to provide controlled diets that included hay and variable rates of sagebrush to steers.

Five crossbred steers (average weight 717 kg) were used in a 5 x 5 latin square experimental design. The steers were fitted with a permanent ruminal cannula of 10.2 cm internal diameter. The steers were housed in partially covered individual pens with concrete floors and had continuous access to hay, water and sulfur salt blocks. Dietary treatments consisted of increasing proportions of sagebrush in a basic grass-hay diet. The hay was of low nutritional quality: 7.4% protein, 68% NDF, and 42% ADF. The sagebrush proportions were 0%, 0.5%, 1%, 3%, and 9% in dry weight basis. Each treatment diet was provided to each steer for a total of 21 days. The experiment lasted a total of 105 days.

Sagebrush leaves were hand-harvested from a private property in Harney County, Oregon. All leaf material was oven-dried and ground to facilitate the preparation of the diet mixtures. Ground sagebrush leaves, mineral and vitamin supplements were mixed with the rumen content and fed immediately before all animals received hay each morning. We collected information on body weight during the last 8 days of each feeding period. The animal feed intake was also recorded throughout each feeding period.

Results

Sagebrush additions significantly increased body weight gains (Fig. 1). The weight gains were successfully modeled by a quadratic regression in which the maximum response seemed to correspond to a dose of 5%-6% of sagebrush in the diet. Higher sagebrush concentrations, like our 9% dose, appeared to have a negative effect in body weight. However, even smaller doses (0.5%-1%) benefitted the steers over the 0% sagebrush diet treatment. Sagebrush additions did not have a significant effect on the voluntary consumption of hay (Fig. 2). Therefore, the gains in body weight can be explained as a result of an improvement in conversion efficiency. We also observed a better body appearance as a result of small amounts of sagebrush in the diets. At this point the specific metabolic effect of sagebrush is not clear and further investigation is needed. Yet, our results suggest that sagebrush in small doses can have a positive effect in cattle growth.

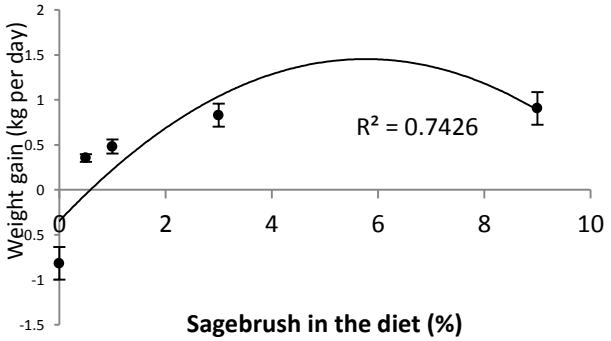

Figure 1. Body weight gains in steers fed different doses of sagebrush mixed with a low quality hay diet. Only average gains and standard error of the mean (bars) are shown. Total number of data points = 25.

Previous studies have reported that sagebrush is a small component (less that 5%) of the diet cattle in southeastern Oregon rangelands (McInnis and Vavra 1987). Our results suggest that cattle instinctively use the small but common sagebrush consumption in order to rectify their needs and balance their diet (Villalba and Provenza 2007). Although more studies are needed to better support this conjecture, our investigation provides evidence that contributes to the growing body of literature emphasizing the view that herbivores "self-medicate" as a means to meet their physiological needs.

Conclusions

Our hypothesis, that appropriately small doses of sagebrush can have beneficial effects on cattle, was supported by our results. The beneficial effect of sagebrush seems related to an improvement of conversion efficiency. However, the specific metabolic effect of sagebrush or its secondary metabolites is yet to be elucidated.

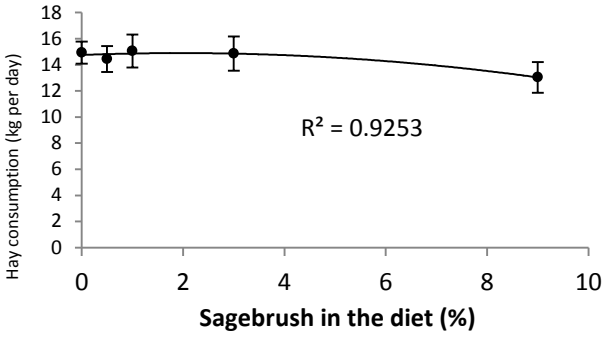

Figure 2. Voluntary hay consumption by steers as affected by the inclusion of different doses of sagebrush in the hay diet.

Acknowledgements

We gratefully acknowledge Dr. Chad Mueller for the use of his experimental steers. Partial financial support was obtained by the Oregon Beef Council and the Agricultural Research Foundation. This work was also partially supported by Mexico's National Council of Science and Technology (CONACYT). A special thanks to Eliether Ureña Armas for her fieldwork assistance.

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Oregon State University

BEEF094

Beef Cattle Sciences

Oregon Beef Council Report

Revegetating sagebrush rangelands invaded by medusahead [1]

Dustin D. Johnson [2] and Kirk W. Davies [3]

Synopsis

Prescribed burning followed by imazapic application was applied to medusahead-invaded rangelands in eastern Oregon to control medusahead in preparation for testing revegetation treatments consisting of native and nonnative (introduced) plant materials. Preliminary results suggest that the most successful revegetation treatment was seeding introduced plant materials one year after prescribed burning followed by imazapic application.

Summary

The objectives of this study were to determine: 1) effective treatments for controlling medusahead and 2) the appropriate plant materials for revegetating medusahead-invaded rangelands. Initial treatments of prescribed burning immediately followed with applications of the preemergent herbicide imazapic (Plateau ®) were applied to three 130 x 165 ft plots at each of five sites in eastern Oregon during the fall of 2010. The same treatment combination was applied to an additional 130 x 165 ft plot at each of the five sites during the fall of 2011. All burned and herbicide treated plots were seeded during the fall of 2011 with either a mix of introduced species, native species, or a combination of introduced and native species. The study also included an untreated reference plot at each of the five sites. Prescribed burning followed with an application of imazapic at a rate of 6 oz/ac substantially reduced medusahead cover and density compared to the untreated reference plots. Fall prescribed burning to remove persistent medusahead litter followed immediately with an application of imazapic at a rate of 6 oz/ac is an effective treatment combination for controlling medusahead and preparing sites for successful revegetation of desirable plants. Initial revegetation success of seeded plants following medusahead control indicated substantially greater establishment of introduced species over native plant materials. Initial results also indicated that reseeding immediately following application of imazapic is not a viable restoration option because of non-target herbicide damage to the seeded vegetation. Instead, our preliminary results suggest initial establishment of seeded plants is best facilitated by using seed mixes predominantly comprised of introduced plant materials and waiting a year after imazapic application before revegetation efforts are attempted.

Introduction

Medusahead (*Taeniatherum caput-medusae* (L.) Nevski) is an aggressive exotic annual grass that decreases biodiversity, degrades wildlife habitat, reduces livestock forage production, and increases fine fuel loads (Davies and Svejcar 2008). Medusahead has invaded at least 5 million acres in the northern Great Basin and an additional 62 million acres are at risk of invasion. Revegetation of medusahead infested rangelands with desirable

1. This document is part of the Oregon State University – 2012 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.

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plants is needed to increase livestock forage production, improve wildlife habitat, restore biodiversity and sustain productivity of adjacent land at risk of medusahead invasion. Medusahead invasion is a particularly challenging management problem because most efforts to revegetate infested rangeland are unsuccessful (Young 1992). Short-term control of medusahead has been accomplished with various treatments. Perhaps the most promising combination of treatments for control of medusahead has been prescribed burning followed by a fall application of imazapic. Davies (2010) demonstrated that introduced perennial grasses could be successfully established after using fire-imazapic treatment to control medusahead, but treatment plots were too small (16.4 by 16.4 ft) to be applicable to large-scale management scenarios encompassing a broader range of environmental conditions. In addition, Davies (2010) did not evaluate the potential use of native plants for revegetation.

Revegetating rangelands invaded by medusahead is constrained by a lack of information to assist land managers in determining 1) the best method to control medusahead and promote revegetation success and 2) the most suitable plant materials to seed after medusahead control. Objectives of this study were to 1) evaluate and demonstrate the effectiveness of prescribe burning followed by imazapic application to control medusahead 2) compare the post-control revegetation success of native plants to introduced plants, and 3) determine if revegetation should occur immediately or one year after medusahead control.

Materials and Methods

Five sites (blocks) in eastern Oregon invaded by medusahead with varying soils, potential natural vegetation, slope, aspect, and elevation were selected for study. Each block consisted of five 130 X 165 ft plots randomly assigned to the different treatments with a 6-ft buffer between treatments. Three of the plots were prescribed burned and then about two weeks later treated with imazapic (Plateau®) during in the fall of 2010. In the fall of 2011, one year after control treatments were conducted, these plots were randomly assigned to be seeded with a mix of introduced, native, or introduced-native species. Also in the fall of 2011, another plot was prescribed burned and treated with imazapic. This plot was immediately seeded with the introduced species mix to determine if seeding can occur without waiting one year after applying imazapic. The fifth plot served as an untreated reference plot at each study site. The introduced species mix included forage kochia, an introduced shrub that is highly palatable and nutritious, crested wheatgrass, and Siberian wheatgrass. The native seed mix consisted of Wyoming big sagebrush, bluebunch wheatgrass, and bottlebrush squirreltail. The introduced-native mix consisted of forage kochia, Wyoming big sagebrush, crested wheatgrass, Siberian wheatgrass, bluebunch wheatgrass, and bottlebrush squirreltail.

Prescribed burns were applied in late September or early October as strip-head fires ignited with drip-torches. After burning, imazapic was applied at a rate of 6 oz/acre. This rate has been used to successful control medusahead in other research projects (Davies 2010; Davies and Sheley 2011). Immediately or one year after imazapic application, depending on treatment, shrubs were broadcast seeded and perennial grasses were drill seeded using a Versa-Drill (Kasco, Inc., Shelbyville, IN, USA). Species were mixed together in equal proportions and seeded at 20 lbs/ac PLS for grasses and 3 lbs/ac PLS for shrubs. This seeding rate was found to be effective for establishing introduced perennial grasses in medusahead infestations that had been controlled with prescribed fire and herbicide application (Davies 2010).

Vegetation cover and density were sampled during the summer of 2012 to determine initial revegetation success of seeded species. Shrub cover was measured by species using the line-intercept method on four, 130-ft transects spaced at 16.4-ft apart. Shrub density was measured by counting all shrubs by species rooted inside 6.5 X 130-ft belt transects placed over the four, 130-ft transects. Herbaceous cover and density were measured by species in 1.3 X 1.6 ft quadrats located at 13-ft intervals along the 130-ft transects, resulting in 10 quadrats per transect and 40 quadrats per treatment plot. The quadrats were divided into 1, 5, 10, 25, and 50% segments to improve accuracy of cover estimations. Because of the potential for adverse effects of forage sampling on relatively young seedlings, forage production and quality will be determined in the third year post-seeding.

Results

Prescribed burning followed with an application of imazapic at a rate of 6 oz/ac reduced medusahead cover and density to <3% and <80 individuals per yd^2, respectively, the second year

following treatment, whereas, the untreated control plots supported medusahead cover and density values of >18 % and 1250 individuals per yd^2 (Figure 1). Figure 2 illustrates the contrast in vegetation between plots receiving no treatment (reference plots) and plots receiving a revegetation treatment of introduced plant species one year after medusahead control using burning and imazapic applied in the fall. Cover of perennial grasses in plots revegetated with introduced plant species was higher than the control plots and plots reseeded with only native plant species or plots seeded directly after imazapic application ($P < 0.05$). Density of perennial grasses in plots revegetated with introduced plant species or a mix of introduced and native plant species was higher than in plots reseeded with native plant species alone the first growing season after seeding ($P < 0.05$, Figure 1). Density of perennial grasses in plots reseeded with a mix of introduced and native plant species (10 lbs./ac native grasses and 10 lbs./ac introduced grasses) was roughly half of the density measured in plots where revegetation was accomplished with introduced plant species alone (20 lbs./ac introduced grasses). Density and cover of perennial grasses in plots seeded immediately following medusahead control with imazapic was substantially lower than in plots seeded one year after imazapic application ($P < 0.05$, Figure 1).

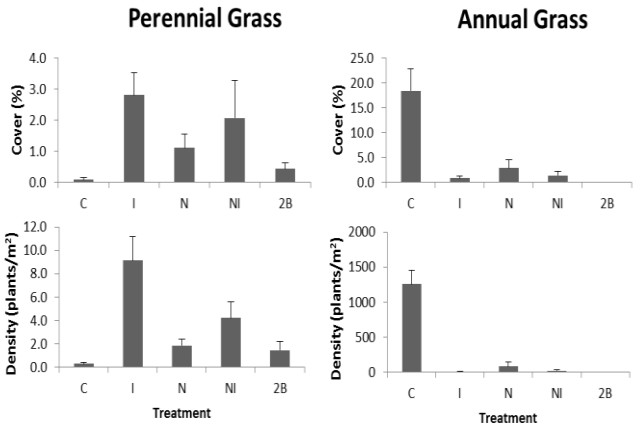

Figure 1. Perennial grass (left panes) and annual grass (right panes) cover and density in plots receiving no treatment (C), revegetation with introduced plant species one year after medusahead control (I), revegetation with native plant species one year after medusahead control (N), revegetation with a mix of introduced and native species one year after medusahead control (NI), and revegetation with introduced plant species immediately following medusahead control (2B).

Figure 2. Illustration of the contrast in vegetation between plots receiving no treatment (untreated plot shown in the left side of photo) and plots receiving revegetation with introduced plant species one year after medusahead control using burning and imazapic applied in the fall (shown in right side of photo).

Conclusions

Initial results suggest fall prescribed burning followed immediately with an application of imazapic at a rate of 6 oz/ac is an effective treatment combination for controlling medusahead. Burning reduces the persistent litter layer that develops on medusahead-invaded sagebrush rangelands. Removing the litter layer prior to herbicide treatment likely serves to increase bare ground which improves soil coverage of imazapic, a preemergent, soil-active herbicide. Likely, without removing litter by burning or some other means (i.e., mechanical), the preemergent activity of imazapic on medusahead, a winter annual grass, would be greatly reduced and control success would be compromised. Prescribed burning and imazapic application also greatly reduced competition from medusahead which provided a more favorable seedbed for revegetation. Initial revegetation success of seeded plants following medusahead control indicated substantially greater establishment of introduced species over native plant materials. Preliminary results also indicate that reseeding immediately following application of imazapic is not a viable restoration option, probably due to herbicide damage to seeded species. Instead, our initial results suggest establishment of seeded plants is best facilitated by using seed mixes predominantly comprised of introduced plant materials and waiting a year after imazapic application before seeding. Preliminary vegetation data reported in this paper are from the first year following revegetation treatments and from

the first or second year following medusahead control, depending on treatment. Vegetation cover and density measurements will be conducted over the next two years (2013 and 2014) and summarized in a final report.

Acknowledgements

This research study was financially supported by the Oregon Beef Council and the USDA – Agricultural Research Service.

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Oregon Beef Council Report

Progress Reports – Rangeland Ecology and Management [1]

Prescribed burning and herbicide applications in the spring to revegetate medusahead-invaded rangelands

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Project Objectives: To determine effectiveness of prescribed burning and various herbicides applied in the spring at controlling medusahead and determine the best treatment for promoting the establishment of seeded perennial grasses.

Project Start Date*:* Fall 2012
Expected Project Completion Date: Summer 2015

Project Status: Six study sites were selected and exclosures were erected around each site during the fall of 2012. The following treatments will be randomly assigned to 20 by 20 ft plots separated by a 3-ft buffer at each site during the spring of 2013: prescribed spring burn, contact herbicide application, pre-emergent herbicide application, combination of spring burning and pre-emergent herbicide application, combination contact and pre-emergent herbicide application, untreated (control) that will be seeded, and a control that will not be seeded. All treated plots will be seeded in the fall of 2013 with a mixture containing crested wheatgrass and native perennial grasses. Vegetation cover and density by species will be measured in each treatment during the summers of 2014 and 2015 to determine treatment effectiveness for controlling medusahead and promoting establishment of seeded perennial grasses.

Modification of livestock and sage-grouse habitat following juniper control in sagebrush communities of eastern Oregon

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Cooperators: Oregon Department of Fish and Wildlife (ODFW), Dr. Mike Borman, Department of Animal and Rangeland Sciences, OSU, and Dr. Dana Sanchez, Department of Fisheries and Wildlife, OSU.

Project Objectives: To monitor vegetation and soil water characteristics through a sequence of multiyear juniper control experiments that started in 2009. The final goal is to accumulate knowledge that would allow for better management of livestock and wildlife while maintaining the ecological integrity of the rangeland.

Project Start Date*:* June 2012
Expected Project Completion Date: June 2014

1. This document is part of the Oregon State University – 2012 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.

Project Status: Cover and density of understory vegetation and juniper were initially assessed during the summer of 2012 in all the selected areas within the Phillip W. Schneider Wildlife Area (PWSWA) in Grant County, OR. Additional exclosures where juniper will be experimentally controlled were also established during the summer of 2012 in areas with contrasting understory characteristics. In these areas, forage production varied from 205 lbs. per acre to 1,633 lbs. per acre. Other initial habitat and forage determinations will be summarized and analyzed in 2013. In 2013, the bulk of the field measurements, including soil water content and vegetation, will continue with the goal of determining habitat changes.

Effects of wolf presence on behavioral and stress responses in beef cattle

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Project Objectives: Determine if cows previously exposed to wolves will remain sensitive to the presence of a canine predator and experience altered behavioral and physiological stress responses.

Project Start Date*:* January 2013
Expected Project Completion Date: March 2013

Project Status: This project is a revised version of the proposal entitled "Conflict stressors, spatial behavior and grazing budgets of cattle" funded by the Oregon Beef Council in the 2010-2011 and 2011-2012 cycles. We identified 50 cows that were officially exposed to wolves (herd located in Idaho), which will be transported to the EOARC Burns in January 2013. These and the EOARC cows (naïve to wolf presence) will be exposed to a series of simulated wolf exposure, and several behavioral and stress-related responses will be measured. We expect to document that if cows previously exposed to wolves will experience a greater increase in aggressiveness and neuroendocrine stress reactions following the simulated wolf presence. These results will demonstrate how wolf predation can cause a substantial stress response in beef cows, which has been shown by Reinaldo Cooke's and other research groups to directly impair cattle health and productivity.

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