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Beef Cattle Sciences

Oregon Beef Council Report

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Oregon Beef Council Report

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Oregon Beef Council Report

Modulation of Milk Fat Synthesis in Dairy Animals: A Nutrigenomics Approach[1]

Massimo Bionaz[2], Fernanda Rosa[3], Shelby Filley[4], and Charles T. Estill[5]

Synopsis

Use of a compound that activates a specific nuclear receptor temporarily increased milk fat synthesis in dairy goats and improved the immune system.

Summary

The long term goal is to put tools in the hand of producers to aid in improving the performance and health of animals by modifying the animal's biology via nutrient-gene interaction. The aim of this study was to evaluate the possibility of changing the milk fat production by nutrigenomic intervention. The objective of this experiment was to evaluate the effects of daily injection of 2,4-thiazolidinedione (TZD), an activator of the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ), on milk fat synthesis and health of lactating dairy goats. For this purpose we used 12 Saanen lactating goats. Six goats received daily intravenous injection of TZD and 6 received injection of saline for 25 days. Besides feed intake (i.e., dry matter intake), body weight (BW), and body condition score (BCS), we measured milk yield, milk components, 20 parameters in blood, and expression of genes in adipose tissue and mammary cells. We did not detect significant effect on BW, BCS, milk component and milk yield but we detected a significant decline in non-esterified fatty acids and β-hydroxybutyrate and increase in glucose in blood of TZD-treated compared to control does. TZD-treated goats also had a lower inflammatory marker haptoglobin, higher immunoglobulin, and better antioxidant potential. We did not detect significant effect on expression of genes in the adipose tissue but we measured a decrease in expression of two key genes for milk fat production in mammary epithelial cells in TZD-treated vs. control goats. Overall, the results indicated that activation of PPARγ by 2,4-thiazolidinedione improved the inflammatory status, the immune system, and the liver and prevented the decline of milk fat production. More data are needed to elucidate the regulation of milk fat production by PPARγ but, overall, the data indicated that there is potential to improve animal well-being via nutrigenomic intervention. Results from the present experiment provide support for investigation on the most potent activator of PPARγ among fatty acids composing the diets of lactating ruminants for nutrigenomic interventions.

Introduction

Butterfat has an important impact on the final price of the milk paid to the dairy producers. The amount of milk fat is determined heavily by the genetics of the animals (e.g., in Jersey cows is

>4.5% while in Holsteins is <4%) but can be modified by diet.

Nutrigenomics is a scientific branch of nutrition that studies how molecules contained in feedstuffs can modify the biology of the organism by changing the expression of specific genes. The study of nutrigenomics is very important because, once deciphered the effect of specific compounds present in the feed on expression of genes, we can use such effect to fine-tune the biology of an organism by increasing (or decreasing) the amount of that specific compound in the diet. Considering milk production, the study of the nutrigenomic effect of compounds in feedstuffs can allow us to have a powerful and relatively cheap means to improve efficiency of production and/or quality of the milk.

It is becoming evident that fatty acids can affect the expression of genes. They can do this by binding and activating proteins in cells that turn on or off specific genes. It is known that fatty acids, especially palmitic, have a positive effect on milk fat production when added into the diet. Part of the milk fat increase is due to the augmented availability of fatty acids from the diet. However, recent data seem to suggest a nutrigenomic effect of certain fatty acid on milk fat synthesis. Data indicated that the fatty acids were partly acting through the peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor controlling expression of genes. This protein plays a very important role in controlling the metabolism of lipid in mammals and seems extremely promising in increasing butterfat. Besides the potential for increasing milk fat, the activation of PPARγ in dairy cows can also improve the overall metabolism and health of the animals (Bionaz et al., 2013). One of the main effects of activation of PPARγ is the general increase in insulin sensitivity. It has been shown in cattle that the increase in insulin sensitivity using a drug known to activate PPARγ agonist, called 2,4-thiazolidinedione, improved the metabolism of post-partum cows and decreased inflammation (Smith et al., 2007, Smith et al., 2009).

Based on all of the above, it appears important to test the hypothesis that PPARγ activation can increase milk fat synthesis and improve animal health. The objective of the present study was to test if the activation of PPARγ *in vivo* increases milk fat and improves health in lactating dairy goats. The test of this hypothesis is essential to move toward on-farm practical application because, once we determine the effect of PPARγ, it is possible to investigate which among the fatty acids present in diet is the strongest activator of PPARγ and use this to obtain the desired nutrigenomic effect.

Materials and Methods

Experimental design

Figure 1 depicts the overall experimental design. In the original funded experiment we proposed to have 2 groups of goat and treat one group with pioglitazone, a known PPARγ agonist in mouse and human. We also proposed to treat the animals for 1 month to test long-term (or chronic) effects of PPARγ activation on milk fat synthesis. In a previous experiment designed to test the role of PPARγ activation on the response to mastitis (Richards et al., 2014, Rosa et al., 2015), we used 2,4-thiazolidinedione, another putative PPARγ agonist, but our *in vivo* data and preliminary *in vitro* data indicated that the 2,4-thiazolidinedione was a very weak PPARγ activator in goats. For this reason, in the present experiment we proposed to use an alternative compound (i.e., pioglitazone). However, in an *in vitro* experiment pioglitazone failed to activate PPARγ in goat mammary cells. Furthermore, we discovered that a metabolite of vitamin A, called 9-*cis*-retinoic acid, enhanced the activation of PPARγ by 2,4-thiazolidinedione in bovine mammary cells (Bionaz et al., 2015). These data suggested that the level of vitamin A in the diet can greatly affect the activation of PPARγ. Despite a weak activation of PPARγ, we obtained some promising results in the prior mastitis experiment (Richards et al., 2014, Rosa et al., 2015). These results were exciting and we wanted to take the opportunity of the upcoming *in vivo* trial to test if the addition of vitamin A would have further activated PPARγ *in vivo* and, if this would have further improved the response of the goats to mastitis induction. Based on the above we decided to change the original experimental design and combine two experiments with a single *in vivo* trial and we decided to feed the animals with adequate amount of vitamins, especially vitamin A. Furthermore, we decided to again use 2,4-thiazolidinedione (**TZD**) instead of pioglitazone, as originally proposed. The objectives of the two experiments were: 1) to test the effect of PPARγ activation on milk fat synthesis and animal health and 2) to test the effect of PPARγ activation on the response to mastitis when vitamin A level in the diet is adequate. To accomplish the first objective we used the funding from Oregon Beef Council. For the

second objective we did not use funding from the Oregon Beef Council. Therefore, in the present report we will present only the data for the first objective, since it fulfills the original aim of the project funded by the Oregon Beef Council.

For the overall project (i.e., objective 1 and 2 combined) we used 12 early lactating Saanen goats (mean±SD; 53.6±16.2 days in milk, 69.2±7.1 kg of body weight [BW], 2.6±0.6 body condition score [1-5 scale]). The goats were housed in individual pens at the Hogg Metabolism Barn at Oregon State University. The goats were randomized by BW, milk yield, and milk components into two treatments (6 goats/group). Ten days of adaptation to the new environment was allowed prior starting the experiment. Animals were fed twice a day. The ration was calculated according to the NRC (2007) for small ruminants in mid-lactation. Animals received twice a day orchard grass hay and alfalfa hay. The ration was supplemented in the AM feeding with an individually calculated amount of a commercial grain goat mix (Kountry Buffet, CHS Inc., Sioux Falls, SD) and a mineral mix (SWEETLIX® Caprine Magnum-Milk Mineral, SWEETLIX®, Mankato, MN). Animals were also drenched once a day (i.e. 10 ml/goat, before AM feeding) with a mix of vitamins (Vitamins and Electrolytes, Durvet). Two kids per goat were left to nurse until 4 days prior intramammary infection (i.e., objective 2). Afterwards, the goats were milked twice a day at 7AM and 7PM in a stanchion using a portable milking machine. At the end of the trial goats were euthanized by rapid intravenous injection of barbiturate (Beuthanasia D®) in order to harvest various tissues (i.e. liver, mammary, and adipose tissue) for gene expression analysis.

In order to assess the long-term effect of PPARγ activation on milk fat synthesis and avoid the effect of mastitis induction, we have left one half of the mammary for each goat untreated (while the other half received intramammary infusion of bacteria to induce mastitis). We used the data from that half during the whole study to aid in fulfilling objective 1. In the following section of materials and methods we will describe procedures to fulfill objective 1, only. After the adaptation period, the goats started to receive daily intravenous injections of 8 mg/kg of BW of 2,4-thiazolidinedione (n=6; **TZD**) or sterile physiological saline (n=6; **CTR**) throughout the whole study (25 days). The daily injection was performed at 10AM.

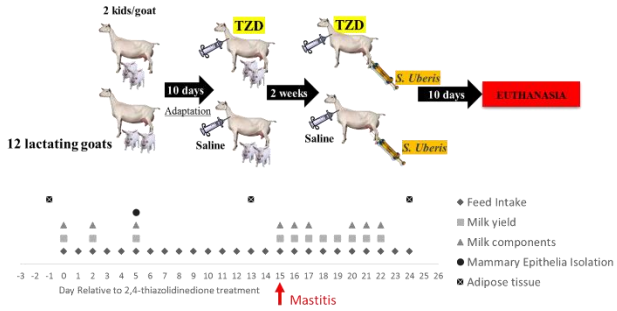

Figure 1. Experimental design

Measurements and sample collection

Goats were milked using a portable milking machine with two claws. In order to avoid cross-contamination, before mastitis induction each goat was milked using one claw; after mastitis induction both claws were used to milk one goat with one claw exclusively used to milk the right half (i.e., the infected half mammary) and the other claw was used to milk the healthy half of the mammary. During the presence of kids, milk yield was recorded by removing the kids at around 7PM and goats were completely milked. The goats were without kids for 12h and were milked again at 7AM of the day after and milk yield was measured. Milk samples were collected for component analysis just before starting TZD injection (time 0) and then at 2, 5, 15, 16, 17, 20, 21, and 22 days of TZD administration. Samples were shipped with a preservative (Bronopol) to the Willamette National Dairy Herd Information Association (Salem, OR) to measure somatic cell count (SCC), lactose, fat and protein percentage. Dry matter intake (DMI) was recorded daily and BW and body condition score (BCS) were recorded weekly throughout the study.

Blood samples were collected prior to the morning milking from the jugular vein. Samples were collected prior starting the TZD injection (time 0) and then at 7, 9, 15, 16, 17, 18, 21, and 25 days into TZD injection. Aliquots of plasma and serum were shipped on dry ice to the Istituto di Zootecnica, Universitá Cattolica del Sacro Cuore, Piacenza, Italy, for metabolic and inflammatory profiling. Plasma samples were analyzed for 19 parameters plus globulin (calculated as total protein –albumin). These include metabolic parameters glucose, cholesterol, urea, creatinine, non-esterified fatty acids (NEFA), triacylglycerol (TAG) and β-hydroxybutyric acid (BHBA); inflammatory-related parameters albumin, haptoglobin, ceruloplasmin,

paraoxonase, myeloperoxidase, total bilirubin, total reactive oxygen metabolites (ROMt), ferric reducing antioxidant power (FRAP) and zinc (Zn); liver status biomarkers gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST/GOT) plus total protein. The analyses were performed following the procedures described previously (Bionaz et al., 2007; Trevisi et al., 2012).

Collection of mammary epithelial cells and adipose tissue

The mammary epithelial cells (MEC) were isolated 5 days into TZD treatment from 250 mL of milk using magnetic beads associated with an antibody that specifically bind to mouse immunoglobulin against a marker of epithelial cells (i.e., cytokeratin 8). We used a KingFisher Duo machine to perform the magnetic sorting. Subcutaneous adipose tissue was collected via biopsy from alternate sides of the tail-head before the morning feeding. The biopsy was performed the day prior starting and at 13 day of TZD injection. Adipose tissue was also collected after euthanasia (i.e., 25 days of TZD injection). RNA was isolated from the above cells and tissues to assess expression of 8 genes known to be target of PPARγ and related to fat synthesis and insulin signaling.

Statistical analysis

Prior to statistical analysis all data except gene expression were arithmetically transformed to have mean at time 0 identical between the two groups. Data were analyzed with the PROC GLIMMIX procedure of SAS 9.2 (SAS Institute, Inc., Cary, NC, USA) to assess the effect of treatment (i.e., TZD or CTR), time (T) and interaction treatment×time. Milk yield data prior to mastitis induction (i.e., 15 days of TZD treatment) were divided by two to account for the milking of two halves and to make the data homogenous through the whole trial, because only one half of the mammary was used for the objective 1 after mastitis induction. Thus milk yield data are reported as half gland per milking. Statistical significance and tendencies were declared at $P < 0.05$ and $0.05 \leq P \leq 0.10$, respectively.

Results

No differences in body condition score or body weight were detected. The daily feed intake was not affected by the injection of the PPARγ activator but there was a tendency for a higher intake in treated does compared to control between 10 and 15 days into the treatment (Figure 2). Milk yield and components were not affected by the treatment (Figure 3); however, a tendency for a different pattern of milk fat % between the two groups was detected before induction of mastitis (day 15 of treatment) with a numerically higher milk fat % two days into injection and a numerically lower amount after 15 days of treatment for the does receiving the PPARγ activator compared to control.

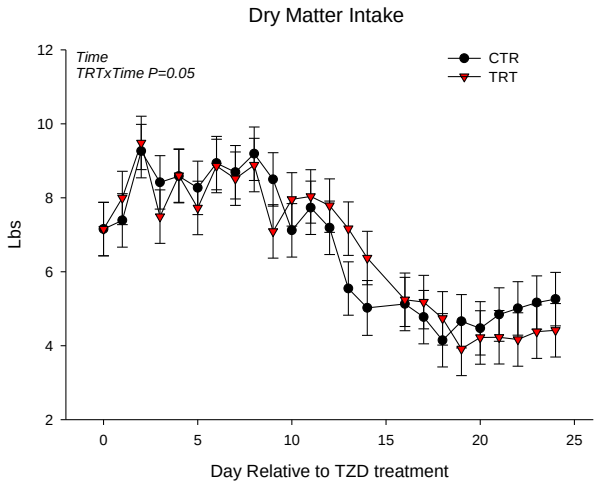

Figure 2. Dry Matter Intake in goats treated daily with the PPARγ activator 2,4-thiazolidinedione (TRT) or saline (i.e., control or CTR). The parameter was overall affected by Time and a tendency for an effect of the treatment over time was also detected. At day 15 a mastitis was induced in the right half of the mammary.

Among the 20 parameters measured in blood we detected differences in 11. From a metabolic standpoint, we detected a large decrease in non-esterified fatty acids (NEFA) and ketone bodies (β-hydroxybutyrate or BHBA) (Figure 4) due to the injection of PPARγ activator. These data are somewhat expected (Smith et al., 2009, Yousefi et al., 2016). PPARγ is known to improve insulin sensitivity in human as it is the target of Avandia (rosiglitazone) the main drug used to treat the insulin resistance disease type II diabetes. Insulin is known to decrease NEFA in blood due to the positive effect on adipose tissue to accumulate fat and decrease release of fat through lipolysis. The decrease of BHBA is a consequence of the decrease of NEFA, since part of BHBA concentration is due to its production by the liver when using NEFA. We also detected a positive effect of the treatment on glucose, which was higher in treated does than controls during the whole trial (Figure 4). Higher amounts of glucose in blood after the treatment with

2,4-thiazolidinedione was also detected in cows treated with the same compound (Smith et al., 2009) or a similar compound (Yousefi et al., 2016). Due to the decrease in NEFA detected, we can assume that we had larger insulin sensitivity in the treated animals. We also did not detect any significant decrease in milk yield or lactose production (Figure 3). When insulin sensitivity increases, given a consistent amount of glucose produced, we should expect a decrease or no change in glucose concentration in blood. Given the above, our data are indicative of larger glucose production by the liver in treated goats.

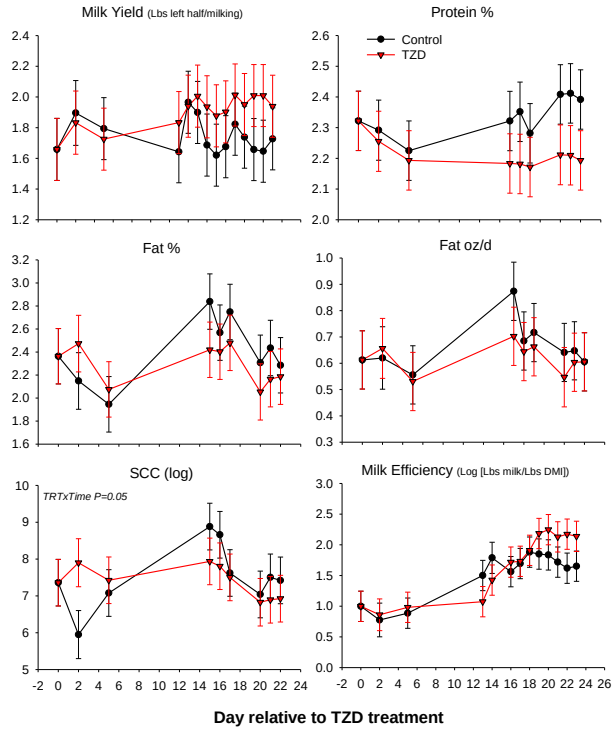

Figure 3. Milk yield, milk components, and milk efficiency in goats treated daily with the PPARγ activator 2,4-thiazolidinedione (TRT) or saline (i.e., control or CTR). The somatic cells tended to be overall affected by treatment through time with a numerical higher SCC at 2 days into the treatment.

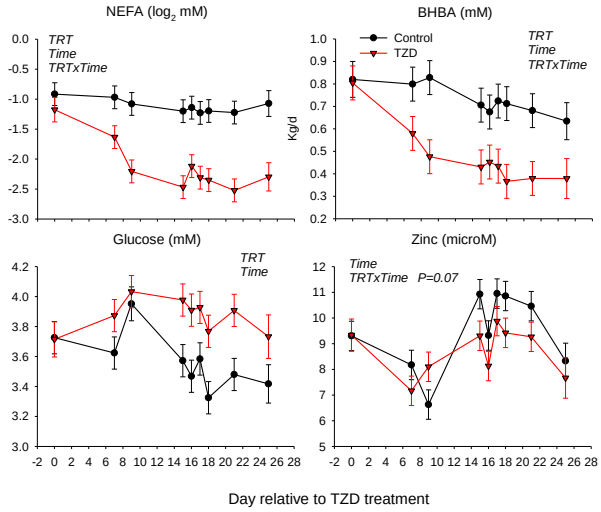

Figure 4. None esterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), glucose, and zinc in blood of goats treated daily with the PPARγ activator 2,4-thiazolidinedione (TRT) or saline (i.e., control or CTR). Parameters overall affected by time, treatment (TRT), and interaction are reported in the graphs, when statistical tendency is detected the P-value is reported.

From inflammatory response/immune system point of view, the data indicate an overall lower inflammation in goats treated with 2,4-thiazolidinedione. This is supported by the overall lower inflammatory marker haptoglobin in blood (Figure 5). The treatment has likely increased the production of immunoglobulin, as measured by globulin (Figure 5). The immunoglobulins are the products of the adaptive immune system. The higher globulin detected is indicative of a better adaptive immune system in goats treated with the PPARγ activator. The liver of the goats was not negatively affected by the injected compounds, as indicated by the liver damage marker GGT which was not higher in TZD-treated compared to control goat (Figure 5).

From an oxidative stress point of view, the goats treated with the 2,4-thiazolidinedione had an overall larger production of peroxides (i.e., ROM), which is a direct indicator of oxidative stress, but also presented a tendency for better antioxidant power, as indicated by the higher ferric reducing ability of plasma (FRAP) (Figure 5). The two parameters appears to contradict each other; however, the measurement of ROM can be difficult and results not always easy to interpret (Kilk et al., 2014). Therefore, our data are likely indicating a tendency for a better antioxidative power in does where PPARγ was activated.

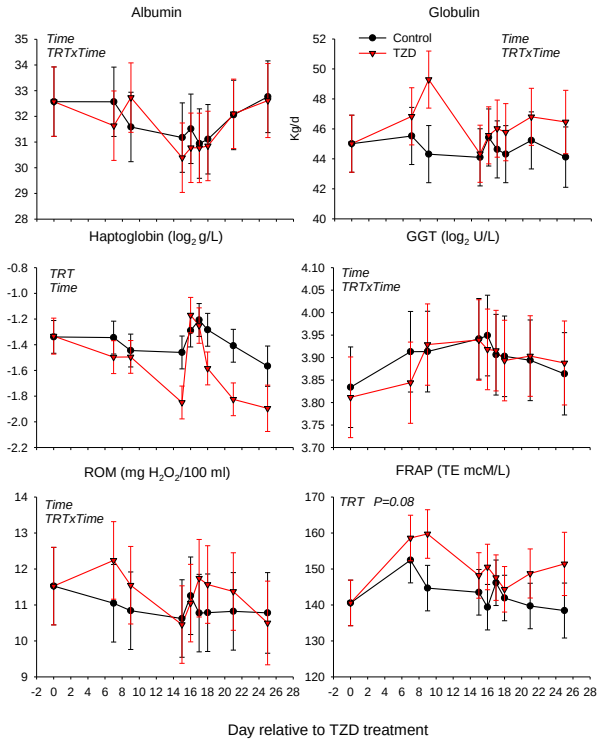

Figure 5. Parameters significantly affected by the treatment to assess the inflammatory conditions (haptoglobin, albumin), immune conditions (globulin), liver damage (GGT or gamma-glutamyl transpeptidase), and oxidative status (ROM or reactive oxygen metabolites and FRAP or ferric reducing ability) in blood of goats treated daily with the PPARγ activator 2,4-thiazolidinedione (TRT) or saline (i.e., control or CTR). Parameters overall affected by time, treatment (TRT), and interaction are reported in the graphs, if tendency was detected the P-value is reported).

Overall the results indicate that the treatment of lactating goats with the putative PPARγ activator 2,4-thiazolidinedione did not affect milk production or the synthesis of milk fat, but significantly affects the availability of NEFA in blood, decreases the inflammatory conditions, improves antioxidant capacity of the animals and, overall, improves the liver status. However, mammary glands use NEFA to produce milk fat. Approx. 50% of the milk fat is produced using fat preformed that derive from diet or from NEFA. There is a positive correlation between NEFA concentration in plasma and milk fat and it has been estimated that NEFA contribute approx.15% to milk fat production (Palmquist, 2006). Given the fact that we did not observe a significant difference in feed intake during the first 10 days of treatment (Figure 2), we should have expected a significant decrease in milk fat production given the large decrease in NEFA, at the least during the first week post-treatment. The fact that milk fat production was not affected by the treatment despite the lower NEFA is indicative of a larger capacity of the mammary gland to produce fat, likely using the *de novo* pathway (i.e., production of fat from scratch instead of taking them up already preformed). Also, the decrease in NEFA detected in goats treated with TZD is indicative of a switch of the adipose tissue toward accumulation of fat. The adipose tissue has a very large expression of PPARγ, at the least 1,000 fold larger than in mammary gland (Bionaz et al., 2013). Thus, we can expect a large activation of PPARγ in adipose tissue in goats treated with 2,4-thiazolidinedione and, likely, larger than in mammary gland. The PPARγ regulates adipogenesis (i.e., the formation of new adipose tissue) and lipogenesis (i.e., the production and accumulation of lipids) in adipose tissue; thus, an activation of PPARγ in this tissue would result, besides a decrease in release of NEFA, an increase in uptake of preformed fat and glucose to form and store lipids. If this is the case, then the mammary gland would have to compete with the adipose tissue on the use of preformed fatty acids to produce milk fat.

In order to test if PPARγ was activated in mammary gland but also in adipose tissue we have measured expression of key genes controlling lipid production in adipose tissue and milk fat synthesis in mammary epithelial cells. The analyses is still ongoing but we have some preliminary data from the adipose tissue collected prior treatment and at 13 and 25 days post-treatment and from the mammary epithelial cells collected 5 days after treatment (Figure 6). More data are needed, but preliminary data indicate that none of the measured genes were significantly affected in adipose tissue. Two key genes, Sterol Regulatory Element Binding Transcription Factor 1 (*SREBF1*), known to play a pivotal role in controlling *de novo* production of milk fat (Bionaz et al., 2015), and Stearoyl-CoA desaturase-1 (*SCD1*) , a recognized key gene in milk fat production (Bionaz and Loor, 2008), were both decreased by 2,4-thiazolidinedione treatment (Figure 6). These data suggested that the 2,4-thiazolidinedione did not affect key genes in fat production in adipose tissue (i.e., not nutrigenomic effect). Lack of effects on genes in adipose tissue is consistent with what was previously observed in cows (Schoenberg and Overton, 2011).. Expression of the two key genes in milk fat synthesis is often associated with milk fat depression. We measured the expression of genes 5 days into 2,4-

thiazolidinedione, looking at the Figure 3, the milk fat production was indeed decreasing in 2,4-thiaszolidinedione treated goats. However, based on the two decreased genes, we should expect a milk fat depression, but we did not observed that. To further test if there was a competition between adipose tissue and mammary gland we still need to section the adipose tissue in order to see if more fat was accumulated and we need also to measure the composition of fatty acids in milk. This will allow us to see if the mammary gland of 2,4-thiazolidinedione treated does had an increased production of *de novo* synthesized fatty acids (i.e., the short-medium chain fatty acids). Preliminary gene expression data do not support such an effect, but more data are needed to make a conclusion. Overall, the mammary gland of goats treated with 2,4-thiazolidinedione was able to maintain the milk fat production despite the apparent competition from the adipose tissue. Assuming that 2,4-thiazolidinedione is a PPARγ activator, the above data indicate that this adaptation was likely driven by PPARγ.

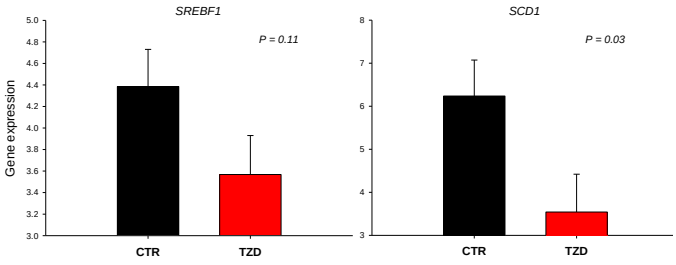

Figure 6. Expression of two key genes controlling milk fat synthesis Sterol Regulatory Element Binding Transcription Factor 1 (SREBF1) and Stearoyl-CoA desaturase-1 (SCD1) was significantly down-regulated in mammary epithelial cells after 5 days of 2,4-thiazolidinedione (TZD) treatment.

Conclusions

In conclusion, the treatment of the goats with a PPARγ activator improved the overall immune system, improved the liver, and strongly decreased NEFA. Furthermore, the activation of PPARγ prevented the decrease in milk fat production expected with the large decrease in NEFA and the decrease in expression of key genes involved in milk fat production. Overall the data are indicative of the potential benefit of activation of PPARγ in dairy animals. These data provide further support for studying the dietary fatty acids that can activate PPARγ. Eventually, we hope this information will provide producers a means to improve animal health using nutrigenomic properties of dietary fatty acids.

Acknowledgements

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Oregon State University

BEEF147

Beef Cattle Sciences

Oregon Beef Council Report

Development of an Enhanced Cattle Embryo Transfer Medium to Improve Pregnancy Rates in Embryo Transfer Recipients[1]

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

Synopsis

Strawing embryos in ETM can be easily merged into the current transfer procedures and would be the most applicable strategy for improving recipient conception rates.

Summary

The specific aim of this research was to evaluate strategies for improving conception rates in embryo transfer recipients that would be compatible with transfer procedures presently used by the industry. The average conception rate for cows used as recipients in nonsurgical transfers with fresh embryos is 61% so there is room to improve this percentage. The period between collecting and transferring the embryos offers a window of time where embryos could be incubated briefly ($\leq$ 2 h) in a culture medium that enhances or stimulates their development prior to transfer. Alternatively, embryos could be strawed in a similar medium thereby transferring both embryo and modified medium to the recipient's uterus. The first experiment was a "proof of concept" undertaking where embryos were incubated immediately after collection in a modified culture medium containing 0 or 100 µg/ml plasmin for 16 h. Although the difference was not significant because the number of transfers was low (n = 48), the conception rate was 12% greater in recipients receiving embryos incubated for 16 h in medium containing 100 vs. 0 µg/ml plasmin. Incubating embryos for 16 h would be difficult to integrate into current transfer procedures, so in the second experiment conception rates were evaluated in recipients receiving embryos incubated for 2 h in medium containing a higher dose of plasmin, 200 vs. 0 µg/ml. The higher plasmin dose for the shorter time had no effect on conception rate and, in fact, conception rates were identical (52%). The third experiment compared conception rates in embryos strawed in either the conventional transfer medium or a new, modified embryo transfer medium containing 200 µg/ml plasminogen. Conception rates were greater in recipients receiving embryos strawed in the new plasminogen-containing medium vs. the conventional medium (48 vs. 37%, respectively) however the difference was not significant because the number of transfers to date are low (n = 40). As the strategy where strawing embryos in the new medium seems the most applicable, more embryo transfers are planned for the coming year.

Introduction

A popular applied reproductive technology used to improve herd genetics and female reproductive efficiency and propagate offspring from elite sire-dam matings in cattle is embryo transfer. An embryo transfer is composed of two separate procedures. The

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

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

Our laboratory has been researching production of the protease, plasminogen activator (PA), by cultured cattle embryos since 1987 (Menino and Williams, 1987). Plasminogen activator secretion is undetectable until the blastocyst stage (Days 6-7 post-insemination), increases during blastocoelic expansion (Days 7-9 post-insemination) and remains elevated throughout and after loss of the zona pellucida or hatching (Days > 9 post-insemination). High levels of PA production were associated with embryos undergoing vigorous development whereas low levels of PA were produced by poorly developing embryos. Plasminogen activator has many roles in embryo development but its earliest role is in activating uterine plasminogen into the active protease plasmin which functions to soften the zona pellucida and allow the embryo to shed it. The zona pellucida is a protein layer produced by and laid around the ovum while it is still in the ovary. It is an important structure in the fertilization process but is eventually shed on Day 10 post-insemination in the cow. If the embryo fails to escape the zona pellucida in the hatching process, it can neither expand and elongate nor make contact with the uterus and will die within the zona pellucida. When exogenous plasminogen is added to cattle embryos in culture, significantly more embryos successfully shed the zona pellucida and the rate of development is similar to embryos developing in the uterus. Therefore, in the formulation of our enriched culture and transfer media, we included either plasmin or plasminogen.

The idea behind including these components was to provide additional plasmin to the embryo for structurally softening the zona pellucida and facilitating zona pellucida loss. Concentrations of plasmin and plasminogen were within physiologic limits for the cow. Besides including plasminogen, the transfer medium was further modified by eliminating the serum component traditionally found in the conventional transfer medium (CTM). Serum is a heterogeneous mixture of a wide array of proteins and many protease inhibitors are present in serum, particularly ones that prevent plasminogen activation and inhibit plasmin proteolytic activity. In place of serum we added 15 mg/ml purified bovine serum albumin (BSA). Bovine serum albumin makes up anywhere from 70-90% of the total protein in the cow's uterine environment depending on stage of the estrous cycle and is widely used as a protein supplement for embryo culture. It also has no effect on conversion of plasminogen to plasmin or plasmin proteolytic activity, unlike the protease inhibitors in serum. For the third and last modification in the formulation of our new enhanced transfer medium (ETM), we replaced the traditional DPBS with a buffered embryo culture medium known as Ham's F-12. Unlike DPBS, which is only a physiologic balanced salt solution, Ham's F-12 is a nutritive medium containing a variety of physiologic biomolecules, e.g., vitamins, sugars and fatty acids, and is the medium of choice for culturing cow embryos.

Materials and Methods

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

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

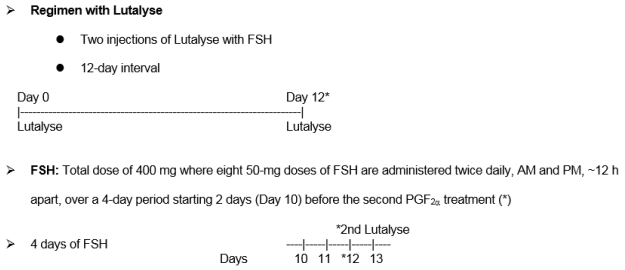

Figure 1. Double injection Lutalyse and FSH protocol for estrous synchronizing and superovulating donor cows for embryo collections.

Experiment 1: Embryos were cultured singly for 16 h in 25-µl microdrops of Ham's F-12 containing 1.5% BSA and 0 or 100 µg/ml plasmin under paraffin oil on 15 x 60 mm plastic tissue culture dishes in a humidified atmosphere of 5% CO_2 in air at 39°C. Embryos were recovered from the microdrops, washed in CTM and aspirated into 0.25 cc straws. Embryos were transferred nonsurgically into timed beef cow recipients within +/- 1 day of the donor's estrous cycle.

Experiment 2: Embryos were cultured singly for 2 h in 25-µl microdrops of Ham's F-12 containing 1.5% BSA and 0 or 200 µg/ml plasmin under paraffin oil on 15 x 60 mm plastic tissue culture dishes in a humidified atmosphere of 5% CO_2 in air at 39°C. As in Experiment 1, embryos were recovered from the microdrops, washed in CTM, aspirated into 0.25 cc straws and transferred nonsurgically into timed dairy cow recipients.

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

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

rectal palpation between equivalent gestational days 45-60.

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

Results

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

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

Experiment 3: A total of 40 embryo transfers were conducted in dairy and crossbred beef cows in Experiment 3. Conception rates were greater in recipients receiving embryos strawed in ETM containing 200 µg/ml plasminogen compared to the CTM (48 vs. 37%, respectively) however the difference was not significant (Table 1).

Conclusions

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

The second strategy was to develop a new embryo transfer medium that would contain plasminogen for the purpose of softening the zona pellucida and BSA in the place of the commonly used cow serum as the nutrient protein source. Cow serum is rich in protease inhibitors and these inhibitors may interfere with proteases, like PA, that are involved in shedding the zona pellucida. The phosphate buffered saline used in the CTM was also replaced with a more nutritive embryo culture medium, Ham's F-12. Although conception rate was greater by 11% in recipients receiving embryos strawed in the new plasminogen-containing medium, ETM, compared to the CTM, the difference was not statistically significant, presumably because the number of transfers was low (n = 40). Strawing embryos in ETM can be easily merged into the current transfer procedures and would be the most applicable strategy for improving recipient conception rates. More embryo transfers are planned for the coming year to continue to compare conception rates in recipients receiving embryos strawed in the new ETM containing plasminogen to those strawed in the CTM.

Acknowledgements

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

16 h incubation[a]		**2 h incubation**[b]		**Transfer medium**[c]	
0 µg/ml plasmin	38%	0 µg/ml plasmin	52%	CTM	37%
100 µg/ml plasmin	50%	200 µg/ml plasmin	52%	ETM	48%

[a]48 transfers; beef cows
[b]54 transfers; dairy cows
[c]40 transfers; beef and dairy cows

Oregon State University

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Beef Cattle Sciences

Oregon Beef Council Report

Impact of Previous Exposure to Wolves on Biological Markers Associated with PTSD in Beef Cattle Following a Simulated Wolf Encounter[1]

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Synopsis

Cows originated from a wolf-experienced herd presented biological evidence suggestive of PTSD after the simulated wolf encounter when compared with cohorts originated from a wolf-naïve herd

Summary

This experiment compared expression of biological markers associated with PTSD in wolf-naïve (n = 10, from Burns, Oregon; **CON**) and wolf-experienced beef cows (n = 10, from Council, Idaho; **WLF**) that were experimentally subjected to a simulated wolf encounter. After a 60-day commingling and adaptation period, CON and WLF cows were allocated to groups A or B (day -1; 5 CON and 5 WLF cows in each group). On day 0, cows from group A were sampled for blood and immediately slaughtered, and samples represented baseline differences between CON and WLF cows. On day 1, cows from group B were exposed in pairs (1 CON and 1 WOLF cow) to experimental procedures. More specifically, cows were sampled for blood, moved to 2 adjacent drylot pens (1 WLF and 1 CON cow/pen) and subjected to a simulated wolf encounter event for 20 min, which consisted of (1) cotton plugs saturated with wolf urine attached to the drylot fence, (2) continuous reproduction of wolf howls, and (3) 3 leashed dogs that were walked along the fence perimeter. Thereafter, another blood sample was collected and cows were slaughtered. Upon slaughter, the brain was removed and immediately dissected for collection of the hypothalamus, as well as 1 longitudinal slice of the pre-frontal cortex, amygdala, and CA1 region of the hippocampus from both cerebral hemispheres. After the simulated wolf encounter, expression of hippocampal *brain-derived neurotrophic factor* mRNA and expression of c-*Fos proto-oncogene* mRNA in hippocampus and amygdala were reduced ($P \leq 0.04$) in WLF compared with CON cows. These are key biological markers known to be down-regulated during fear-related psychological disorders including PTSD. Hence, cows originated from a wolf-experienced herd presented greater biological evidence suggestive of PTSD after the simulated wolf encounter compared with cohorts originated from a wolf-naïve herd.

Introduction

With the reintroduction of grey wolves into the Yellowstone National Park, the dispersion of wolf packs into agricultural lands in the northwestern United States markedly increased (Larsen and Ripple, 2006). Wolves started to inhabit and hunt in livestock grazing areas, escalating the incidence of cattle-wolf interactions and cattle

1. This document is part of the Oregon State University – 2016 Oregon Beef Council Report. Please visit the Beef Cattle Sciences website at http://beefcattle.ans.oregonstate.edu.\
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predation by wolves in the region (Idaho Department of Fish and Game, 2015; Oregon Department of Fish and Wildlife, 2016). Although the economic implication of predators on livestock systems is primarily associated with animal injury or death, these parameters are not the only negative impacts that wolf predation causes to beef cattle systems.

The mere presence of predators alters stress physiology and behavior parameters of the prey, particularly if the preyed animal was already exposed to similar predation episodes (Boonstra, 2013). Accordingly, research from our group suggested that presence of wolves increases excitability and fear-related physiological stress responses in cows previously exposed to wolves, but not in cows unfamiliar with this predator (Cooke et al., 2013). More specifically, Cooke et al. (2013) subjected beef cows from wolf-naïve and wolf-experienced origins to a simulated wolf encounter, which included olfactory, auditory and visual stimuli for 20 min. The wolf simulation process increased aggressiveness, body temperature, and plasma cortisol concentration in wolf-experienced cows but not in wolf-naïve cows, and all these responses are known to directly impair cattle welfare and productivity. In addition, these results suggest that wolf presence alters psychological parameters in wolf-experienced cows due to adverse memories from previous predation episodes, leading to behavioral and physiological changes comparable PTSD. To test this latter rationale, this experiment compared expression of neural and blood biomarkers associated with PTSD in wolf-naïve and wolf-experienced beef cows that were experimentally subjected to auditory, olfactory, and visual stimuli designed to simulate a wolf encounter.

Materials and Methods

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

Animals management

Multiparous, non-pregnant, non-lactating Angus-crossbred cows from EOARC (**CON**; n = 10) and from a commercial cow-calf operation (**WLF**; n = 10) near Council, Idaho were used. Both locations used domestic herding dogs to move cattle across pastures or to the handling facility, although no work dogs were present at the EOARC during the experimental period. The CON cows were selected from the EOARC mature cowherd. The EOARC herd is reared and maintained near Burns and Riley (Oregon) and to date no known wolf packs exist nor wolf-predation episodes have occurred in this region (Oregon Department of Fish and Wildlife, 2016). Hence, CON cows were considered naïve to wolf presence and predation. The WLF cows were selected from the commercial operation located near Council, Idaho. This region (McCall-Weiser Wolf Management Zone) includes active wolf packs (Idaho Department of Fish and Game, 2015). The herd from which WLF cows were selected have experienced multiple confirmed wolf predation episodes from 2008 to 2015 when grazing summer pasture allotments (USDA-APHIS, Idaho Wildlife Services, Boise, Idaho); although, none of the experimental WLF cows had been directly predated or injured by wolves. Therefore, WLF cows were considered experienced with wolf presence and predation episodes.

The WLF cows were transported to the EOARC 60 days prior (day -60) to the beginning of the experiment (day 0). During this period (day -60 to d -1), CON and WLF cows were commingled and maintained in a single meadow foxtail dominated pasture harvested for hay the previous summer, and had ad libitum access to meadow-grass and mineral-vitamin supplement. All cows were also individually processed through the EOARC handling facility, but not restrained in the squeeze chute, once a week from d -60 to -5 to acclimate WLF cows to the EOARC personnel and facilities. On day -1, cows were ranked within previous wolf exposure status (CON and WLF) by temperament score by the same single technician), and allocated to 2 groups of 10 cows each (5 CON and 5 WLF cows in group A and group B) in a manner that groups had similar temperament. All cows from group A were assigned to experimental procedures on day 0, which did not include a simulated wolf encounter to represent baseline differences between CON and WLF cows in the biomarkers evaluated herein. All cows from group B were assigned to experimental procedures on day 1, which included the simulated wolf encounter

Experimental Procedures

Group A. On day 0, cows from group A were moved to a single drylot pen with free choice hay and water, while cows from group B remained on pasture. Cows from group A were individually processed for

blood collection, and immediately slaughtered. Cows from group A that were yet to be processed and all cows from group B were maintained, respectively, 300 and 500 yards from the processing-slaughter site to prevent cows from perceiving the sampling and slaughter process.

Group B. On day 1, cows from group B were also moved to a single drylot pen with free choice hay and water. Two cows, being 1 CON and 1 WOLF, were selected and concurrently processed for blood collection. After blood collected, these 2 cows were immediately assigned to the simulated wolf encounter described by Cooke et al. (2013). More specifically, cows were moved to 2 adjacent drylot pens separated by a fence line (1 WLF and 1 CON cow in each pen). Pens were located 100 m from the handling facility, and had no feed or water source. After arrival in their respective pens, CON and WLF cows were immediately subjected to a simulated wolf encounter for 20-min. Specifically, wolf urine (Harmon Wolf Urine Scent; Cass Creek, Grawn, Michigan) was applied to 12 cotton plugs (Feminine care tampons; Rite Aid, Camp Hill, Pennsylvania), and plugs were attached to the drylot fence line (6 plugs /pen) before any experimental procedures on day 1. After cows were settled within each dry lot pen, wolf howls previously recorded from the wolf packs residing in Wallowa County, Oregon, were continuously reproduced using a stereo system (S2 Sports MP3 CD/Radio Boombox; Sony Corporation of America, San Diego, California); cows had no visual contact with the stereo system. Also, 3 dogs were conducted using a leash by 2 technicians outside the drylot perimeter fence during the entire 20-min simulation. The dogs were 2 adult German Shepherd females to represent adult wolves, and 1 adult Border Collie × Alaskan Malamute female to represent a young wolf. Immediately after the simulated wolf encounter, another blood sample was collected and cows were slaughtered. Cows from group B that were yet to be processed were maintained, respectively, 300 yards from the site were sampling, simulated wolf encounter, and slaughter were performed to prevent cows from perceiving these procedures. All cows were sampled, exposed to the simulated wolf encounter, and slaughter in pairs (1 CON and 1 WLF cow).

Slaughter. All cows were slaughtered using the same procedures and in the same site. Cows were individually restrained and rendered unconscious using a non-penetrative captive bolt stun gun (Cash Special 0.25 Caliber Non-Penetrating Heavy-Duty Stunner with 3.5 grain Power Load; Accles & Shelvoke Inc., West Greenwich, Rhode Island) to prevent excessive brain structural damage. Once unconscious, cows were exsanguinated by incision of the ventral aspect of the throat or neck transecting skin, muscle, trachea, esophagus, carotid artery and jugular vein with a sharp knife with a 10-in rigid blade. Following exsanguination, the brain was removed and immediately dissected for collection of the hypothalamus, as well as 1 longitudinal slice of the pre-frontal cortex, amygdala, and CA1 region of the hippocampus from both cerebral hemispheres.

Sample analysis

Brain tissue samples. Total RNA was extracted only from tissue samples using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, California). Besides the hypothalamus, samples from the left and right hemispheres were combined for RNA extraction. Extracted RNA (200 ng) was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems, Foster City, California). Real-time PCR was completed using the Fast SYBR Green Master Mix (Applied Biosystems) and gene-specific primers (20 pM each) with the StepOne Real-time PCR system (Applied Biosystems), A portion of the amplified products were purified with the QIAquick PCR purification kit (Qiagen Inc., Valencia, California) and sequenced at the Oregon State University - Center for Genome Research and Biocomputing to verify the specificity of amplification. All amplified products represented only the genes of interest.

Blood samples. Total RNA was extracted from blood samples using the PAXgene Blood RNA Kit (Qiagen). Assessment of quantity and quality of isolated RNA, reverse transcription (120 ng of extracted RNA), real-time RT-PCR with gene-specific primers (20 pM each;), and specificity of amplification were performed as described for brain tissue samples.

Statistical analysis

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

Results

Results from our previous research (Cooke et al., 2013) suggested that exposing wolf-experienced cows to a simulated wolf encounter elicited behavioral and physiological changes comparable to PTSD symptoms. However, the same outcome was not detected in cows unfamiliar with this predator, perhaps due to the lack of adverse and fear memories from past predation episodes. Comparable fear-related stress models were adopted to investigate PTSD biological markers in rodents (Ressler et al., 2011; Zhang et al., 2015; Dong et al., 2016), including predator-scent stress using feline urine (Kozlovsky et al., 2007). Collectively, these and other research evaluating PTSD in human and rodent models identified biological markers expressed in blood cells, as well as hypothalamus, amygdala, pre-frontal cortex, and CA1 region of the hippocampus (Kozlovsky et al., 2007; Le-Niculescu et al., 2011; Sherin and Nemeroff, 2011). Together, these regions constitute the brain "fear network", and have been directly implicated in PTSD-like stress responses (Gorman et al., 2004; Bremner et al., 2006; Sherin and Nemeroff, 2011). To our knowledge, no other research has evaluated similar parameters in beef cattle; hence, this experiment focused on PTSD-related biomarkers established in the rodent and human literatures.

Table 1. Expression of amygdala genes.

Item	WLF	CON	*P*=
Caspase 3			
Group A	2.56	2.01	0.39
Group B	2.34	3.26	0.16
Caspase 9			
Group A	1.72	1.27	0.14
Group B	1.13	1.48	0.24
Carnitine palmitoyltransferase 1B			
Group A	3.11	3.25	0.87
Group B	1.56	2.26	0.42
Down syndrome cell adhesion molecule			
Group A	3.58	3.16	0.74
Group B	5.00	5.52	0.69
c-Fos proto-oncogene			
Group A	2.76	1.31	< 0.01
Group B	1.31	2.41	0.02

Across all blood-brain biomarkers associated with PTSD evaluated herein (Tables 1, 2, 3, and 4), only expression of hippocampal *brain-derived neurotrophic factor* and expression of *c-Fos proto-oncogene* in CA1 hippocampus and amygdala differed between WLF and CON cows after the simulated wolf encounter. Nevertheless, *c-Fos* mRNA expression is down-regulated by fear stimulus (Day et al., 2008), and was considered the top biomarker for anxiety disorders including PTSD among a multitude of brain–blood genes evaluated by Le-Niculescu et al. (2011). Expression of *brain-derived neurotrophic factor* mRNA in CA1 hippocampal region was also reduced in rats exposed to a predator-scent stress model (Kozlovsky et al., 2007), which was accompanied by increased excitability and greater plasma corticosterone concentrations comparable to our previous findings in wolf-experienced cows (Cooke et al., 2013). The reason to why expression of these genes in cows from group A was greater in WLF vs. CON cows is unknown, as cows from group A were managed similarly prior to slaughter. Nevertheless, these outcomes further illustrate how the simulated wolf encounter differentially changed expression of PTSD biomarkers between wolf-experienced and wolf-naïve cows.

Neural responses to fear appear to be initiated in the amygdala, with direct stimuli from the hippocampus when fear-related memories are present, followed by projections into other brain region and across the blood-brain barrier (Gorman et al., 2000). This latter rationale may help explaining why differences between WLF and CON cows in group B were only noted in amygdala and hippocampal samples; perhaps sampling schedule adopted herein was not appropriate to detect similar outcomes in hypothalamic, pre-frontal cortex, and blood samples. It is also important to note that cattle temperament, body temperature, and plasma cortisol concentrations were not evaluated due to limited statistical power, which needed at least 50 WLF and 50 CON cows based on the G*power 3 software and outcomes from Cooke et al. (2013). Yet, the main goal of the present experiment was to focus on mRNA expression of established PTSD biomarkers in human and rodent literature, and provide novel information regarding the impacts of wolf predation on beef cattle welfare

Conclusions

Collectively, results from this experiment indicate that the simulated wolf encounter down-regulated expression of hippocampal *brain-derived neurotrophic factor* and *c-Fos proto-oncogene* in hippocampus and amygdala, which are key biological markers of psychological disorders

elicited primarily by fear. These outcomes are corroborated by comparable research with rodents (Kozlovsky et al., 2007; Day et al., 2008), and suggest that altered behavior and physiological changes in wolf-experienced cows exposed to the simulated wolf encounter by Cooke et al. (2013) can be associated with PTSD symptoms. Hence, the presence of wolf packs near cattle herds may negatively impact beef production systems via predatory activities and subsequent death and injury of animals, as well as by inducing PTSD-like responses that impair cattle welfare and productivity when packs are in close proximity to previously predated herds.

Table 2. Expression of genes from the CA1 region of the hippocampus

Item	WLF	CON	*P*=
Adenylate cyclase activating polypeptide 1			
Group A	18.0	39.9	0.33
Group B	146.5	157.6	0.62
Brain-derived neurotrophic factor			
Group A	12.6	36.9	0.13
Group B	107.7	146.7	0.02
Down syndrome cell adhesion molecule			
Group A	2.00	2.50	0.40
Group B	5.96	6.46	0.41
c-Fos proto-oncogene			
Group A	4.60	2.76	< 0.01
Group B	1.84	3.00	0.04
Neurotrophic tyrosine kinase receptor type 2			
Group A	2.09	1.95	0.65
Group B	3.14	3.40	0.40
Telomeric repeat binding factor 1			
Group A	2.46	1.95	0.17
Group B	1.30	1.55	0.49
Telomeric repeat binding factor 2			
Group A	1.74	1.57	0.39
Group B	1.52	1.61	0.65

Acknowledgements

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Table 3. Expression of genes from the pre-frontal cortex and hypothalamus

Item	WLF	CON	*P*=
Pre-frontal cortex			
Adenylate cyclase activating polypeptide 1 R1			
Group A	1.64	1.51	0.68
Group B	1.73	1.85	0.73
Heat shock protein 1A			
Group A	2.48	1.81	0.58
Group B	2.64	3.99	0.25
X-box binding protein 1			
Group A	3.98	3.73	0.84
Group B	2.88	3.30	0.74
Hypothalamus			
Adenylate cyclase activating polypeptide 1			
Group A	2.03	2.38	0.49
Group B	1.47	1.39	0.88

Table 4. Expression of genes in blood cells

Item	WLF	CON	*P*=
ATPase H+ transporting accessory protein 1			
Group A	1.34	1.32	0.83
Group B			
Pre-simulation	2.01	1.57	0.13
Post-simulation	2.47	2.32	0.59
c-Fos proto-oncogene			
Group A	2.30	1.68	0.09
Group B			
Pre-simulation	2.64	1.67	0.19
Post-simulation	3.38	2.92	0.52

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Beef Cattle Sciences

Oregon Beef Council Report

Progress Reports – Animal Sciences[1]

Feeding immunostimulant ingredients to optimize health and performance of receiving cattle

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Project Objectives: Evaluate the impacts of immunostimulant ingredients (Omnigen-AF® or Livestock Stress Formulas) on performance, health and physiological variables of receiving cattle.

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

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

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

- Control: No immunostimulant supplementation (**CON**).

- Omnigen-AF®: Supplementation with Omnigen-AF® (22 g/steer daily; Prince Agri Products, Quincy, IL) from day 0 to 30 of the experiment (**OMN**).

- Livestock Stress Formulas: Two capsules of Stocker Immune Primer Release with trace minerals (Ramaekers Nutrition; Santa Cruz, CA) on d 0, in addition to 15g/steer daily of the Stocker Preconditioned Premix (Ramaekers Nutrition) from day 7 to 30 of the experiment (**LSF**).

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

Steers received a free-choice receiving diet based on mixed alfalfa-grass hay, cracked corn, and soybean meal with a forage:concentrate ratio of 60:40 from day 0 to 15, 40:60 from day 16 to 40, and 30:70 from day 41 to date

(as of 11/11/16). Feed intake was recorded daily by measuring offer and refusals from each pen. Blood samples were collected on days 0, 3, 7, 10, 14, 21, 31, 42, 56, and 73 (to be collected) of the experiment, and will be evaluated for immunological and metabolic parameters.

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

To date (as of 11/11/16, day 56 of the experiment), no differences were detected for average daily gain (P = 0.69; Table 1) and body weight change (P = 0.99; Figure 1a). Also, no treatment differences were detected ($P \geq$ 0.36) for health parameters (Table 2 and Figure 1b). Upon conclusion of this experiment (December 2016), complete health and performance assessment will be conducted, and lab work will begin. We expect to provide a final report in the Spring of 2017.

Table 1. Growth and intake parameters during the initial 56 days of the experiment.

Item	CON	LSF	OMN	SEM	*P*-value
Average daily gain (day 0 to 56), lbs/d	2.76	2.64	2.72	0.10	0.69
Feed intake, lbs of dry matter/day					
Hay	6.50	5.92	6.25	0.58	0.78
Concentrate	8.24	8.22	8.40	0.16	0.69
Total	14.74	14.14	14.65	0.69	0.80
Feed efficiency (lbs gain/lbs feed intake)	0.187	0.179	0.186	0.008	0.72

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

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

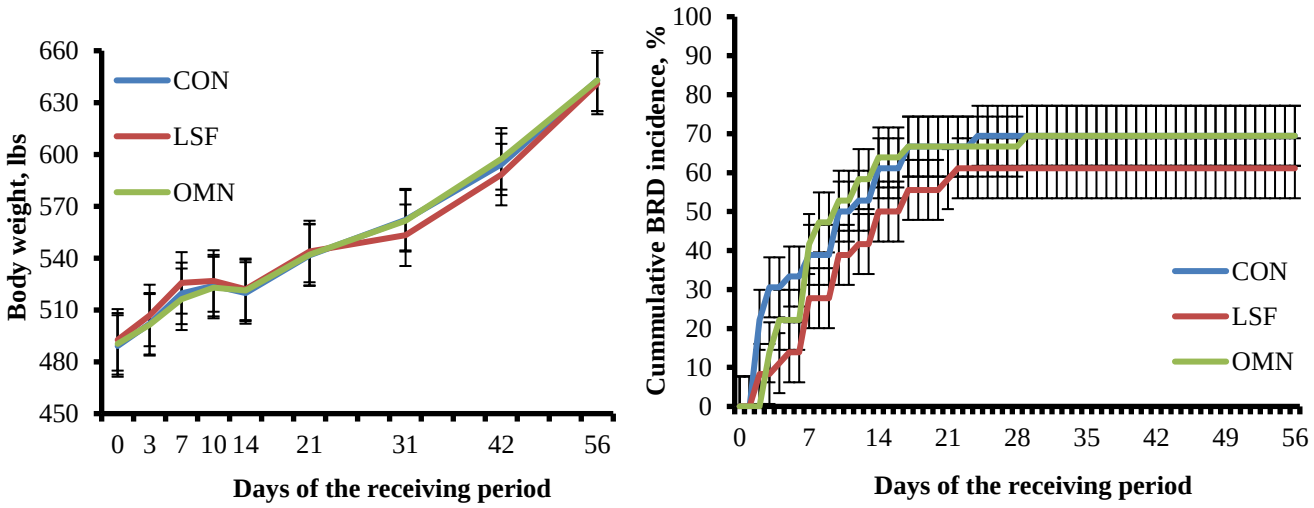

Figure 1. Growth rate (Panel A) and cumulative incidence of BRD symptoms that required antimicrobial treatment (Panel B) during the initial 56 days of the experiment. No treatment differences were detected ($P \geq$ 0.66).

Peripartal Vitamin E Injections Prevent Diseases in Early Lactation Dairy Cows

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Project Objectives: The objective of the proposed study to determine whether subcutaneous injections of vitamin E during the transition period will prevent diseases of multiparous dairy cows.

Project Start Date*:* October 2015

Expected Project Completion Date: October 2017

Project Status: We are currently working on getting additional funding to start the experiment. The results will be published as update 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.

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

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

Project Start Date: Fall of 2016
Expected Project Completion Date: Fall of 2017

Project Status: We are currently working on arrangements to utilize the weaned beef calves from the Oregon State University Soap Creek ranch in an 8 week back ground feeding period before sale to a commercial feedlot. During this 8 week back grounding period, calves will be divided into pens of 5 calves each, balanced by body weight and sex, and fed Se-fortified alfalfa hay. Pen is the experimental unit and multiple animals within the pen provide multiple measurements of treatment response. Three pens of each treatment are needed for replication (n=3). **Group 1 (No-Se forage control):** 3 pens of calves will be fed non-Se fortified alfalfa hay as the major portion of the ration plus a mineral supplement containing 120 mg/kg Se from Na-selenite. **Group 2 (Medium-Se forage):** 3 pens of calves will be fed alfalfa hay fertilized with 45 g Se/ha plus a mineral supplement containing 120 mg/kg Se from Na-selenite. **Group 3 (High-Se forage):** 3 pens of calves will be fed alfalfa hay fertilized with 89.9 g Se/ha plus a mineral supplement containing 120 mg/kg Se from Na-selenite. Calves will be maintained on their respective diets for 8 weeks. We will collect feces at the beginning and end of the 8 week feeding period. Feces will be collected directly from the rectum for fecal flotation to determine the number of ova/gram of feces (McMaster's technique). This test will be performed in the Veterinary Diagnostic Laboratory at Oregon State University. In addition, feces will be submitted for an ELISA assay for determination of *Haemonchus contortus* incidence. Whole blood samples will be collected from the calves every two weeks to determine the effectiveness of Se-fortified alfalfa in raising whole-blood Se levels in the calves and to correlate whole-blood Se levels with efficacy of parasite control.

Evaluation of Biological Deterrents to Manage Wolf Movements and Minimize Cattle Stress

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Project Objectives: To evaluate the relative effectiveness of biological deterrents (commercially available wolf urine, freshly collected and preserved wolf scat from dominant or mixed individuals) for preventing wolves from crossing a trespass line to acquire food bait in a controlled setting. It has been speculated that creating a trespass line with the urine and feces of unfamiliar wolves could be used to establish an artificial territory which would deter other wolves from hunting or feeding within the protected area. However, to date, potential biological deterrents have been less subject to scientific test than other anti-predator strategies (e.g. fladgery) and those studies that do exist have often occurred in settings where the presence and status of wolves in the area could not be assured. Conducting this research in captivity will allow us to know the number and identity of wolves present, the prior feeding schedule of those wolves (relative hunger/motivation at time of trespass), and provide greater control over the testing area than would be possible in field settings. If successful, biological deterrents could provide a sustainable, environmentally sound, and less costly predator deterrent option. If biological methods prove to be unsuccessful in preventing trespass, this would signal a need for caution when studying or applying these methods in farm or ranch settings where livestock could be at risk. This scientific assessment is especially important given commercially available predator urine, including wolf urine, is already heavily marketed for these purposes.

Project Start Date*:* April of 2016
Expected Project Completion Date: August of 2017

Project Status: Phase 1, obtaining biological deterrent samples, has been completed: With the help of our collaborating sites, Wolf Park located in Battle Ground, IN and California Wolf Center located in Julian, CA, we collected over 200 pounds of fresh wolf scat from known wolves between the months of April and August, which was labeled, sorted into ‘dominant wolf’ and ‘mixed status wolf’ categories and immediately frozen upon collection. In August all frozen samples were shipped overnight to the alternative site for experimental testing. Wolf urine was obtained from a commercial source, which allowed us to better evaluate the product currently being used in applied settings- both alone and in combination with fecal samples from known individuals and with known preservation methods prior to use.

Phase 2, Short-Term Assessment of Artificial and Biological Deterrents on Wolf Behavior: Captive wolves at both facilities are currently undergoing testing. We have already completed deterrent setups at both testing facilities (located in CA and IN) and trained study personnel on data collection procedures. Wolves will receive four treatments: Control (water died with food coloring to match the color of the urine), commercially available wolf urine, commercially available wolf urine + unfamiliar dominant wolf scat, commercially available wolf urine + unfamiliar mixed rank wolf scat. Two deterrent areas are present within each enclosure. During the first week- baseline- the control stimulus was placed along all trespass lines, leaving the carcass meat (bait) behind each line unprotected. On all subsequent weeks, one of the three deterrents will be applied to one trespass line, in efforts to slow or deter the removal of fresh bait by resident wolves over a 7-day period. The alternate deterrent area within each enclosure will be set up as a paired control condition. Locations will be counterbalanced and staff will be blind to the testing conditions. Data will be collected twice a day indicating the presence, disturbance or absence of the bait behind each trespass line. Trespass events will also trigger a motion-activated camera. This video will allow us to evaluate behavioral responses of the wolves towards the biological samples, and trespass related behaviors. This will be repeated over four cycles, replicated across seven wolf enclosures/packs.

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

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

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

Project Status: the PI received notification of funded project in September 2016. For this reason, the project has not yet started but some preliminary data were produced. We tested the use of a plasmid in measuring the activation of the nuclear receptor peroxisome proliferator-activated receptor by inserting the plasmid in immortalized bovine mammary cells and treated the cells with a specific activator. The activation of the nuclear receptor was assessed by measuring increase in fluorescence activity by a robotic microscope. The results were compared to a gold standard (i.e., luciferase assay). The sensitivity of the plasmid coding for fluorescent proteins was similar to the gold standard. We have also successfully performed liver biopsies in 2 culled cows. In one cow two liver biopsies were performed 2 weeks apart and the cow was not negatively affected by the procedure. We successfully obtained cells from the liver biopsy. We are waiting for the availability of the cows to start the experiment. It is anticipated that we will start to perform biopsy, isolate hepatocytes, and collect blood from transition cows on February 2017. Sebastiano Busato, a master student that obtained the prestigious Eckelman scholarship will join my laboratory this coming January and he will be leading this project. He worked in my lab for 4 months from February to April 2016 and he has experience in all the techniques required for this experiment.

Oregon State University

BEEF145

Beef Cattle Sciences

Oregon Beef Council Report

Development of a Forage Value Index for Ryegrasses[1]

Troy W. Downing[2]

Synopsis

Evaluating perennial ryegrasses for performance and milk production potential.

Summary

The objective of this study is to develop a ryegrass forage value index for grass cultivars for milk production in Oregon. Energy from pasture is the result the digestion of soluble and structural carbohydrates, proteins, starch and fats. Net energy of lactation (NEl) is the estimated feed energy available for maintenance and milk production after digestive and metabolic losses of all the feed components. Twenty perennial ryegrasses were planted in the fall of 2014 in replicated plots of three complete randomized mixed blocks. The seed was donated by participating grass seed companies. Plots were harvested six times annually for two years and sent to a lab for analysis. In 2015, the variety Trojan had the highest dry matter yield all of twenty varieties and yielded 15,085 lbs. DM per acre. That same year the lowest production variety was Oroverde at 12,503 lbs. DM per acre. Table 2 shows DM produced for each of the twenty cultivars by cutting in 2016. This year the top DM producer was Alto AR37 at 17,393 lbs. DM per acre and the lowest in year two was Albion at 14,340 lbs. DM per acre. Varieties were ranked for estimated milk production using an index based on energy (NEl). Estimated milk production per acre ranged from 284 cwt. to 241 cwt. for the varieties tested. This 43 cwt difference or range could be estimated to be worth from $600-800 dollars and acre difference in total milk produced potentially.

Introduction

As a dairy farmer or beef producer in Oregon it has been difficult to find information on the expected performance differences between forage cultivars. As an industry, the forage grass seed industry has had difficulty over time explaining the economic merit or value of an individual grass cultivar to the livestock industry. Often producers renovate pastures based on seed availability and perceived adaptability with no real appreciation for the genetic selection, testing and breeding efforts that went into developing that cultivar.

In both the beef and dairy industries, livestock breeders have become use to using bull proofs to identify individual bull proofs based off an index system (AIPL, 2012). A similar bull proof process has also been successful in New Zealand and in the Republic of Ireland. However, interest in developing a process for creating a forage value index for individual grass cultivars is beginning to gain interest around the world. At this time, there is not a forage value index in place in the United States.

In Ireland, researchers developed a program to develop an economic ranking index for Irish perennial ryegrasses (McEvoy, 2011). The economically important traits selected in their system were spring, midseason and autumn grass dry matter yield, grass quality, and sward persistency. Like all indexes, each parameter in the index had a different weight based on economic importance.

In New Zealand, researchers have developed an economically based (forage value index) FVI that ranks cultivars according to their overall value to a dairy farm business. This FVI attempts to take into account all the major plant traits that drive the productivity of the dairy farms (Chapman et al., 2016). There are substantial regional differences in New Zealand. This is a major project that has specifically looked at genotype x environment interactions and is used to delineate performance differences in four major regions throughout the country.

According to New Zealand National Forage Variety Trial data, since 1991, the trend for genetic gain in perennial ryegrass has been greatest for summer pasture production (+24lbs DM/acre/year), followed by autumn (+18 lbs. DM/acre/year), winter and late spring (both at +5 lbs. DM/acre/year). Genetic gains in total production of 50-55 lbs. DM/acre/year, or 1,000 lbs. DM/acre since 1990 (Chapman, 2011).

The objective of this study is to develop a Forage Value index for grass cultivar ranking in Oregon. Quality measurements weighted for energy is the driver of this index as energy is the single most limiting factor for milk production or growth in high quality ryegrass pastures. Energy from pasture is the result the digestion of soluble and structural carbohydrates, proteins, starch and fats. Net energy for lactation (NEl) is the estimated feed energy available for maintenance and milk production after digestive and metabolic losses. Included in the objectives of this project is to estimate milk production and beef gain potential differences between the tested cultivars on a per acre basis.

Materials and Methods

Twenty ryegrasses were planted in the fall of 2014 in replicated plots of three completely randomized mixed blocks in plots that measured 4'x10'. The seed was donated by participating grass seed companies. All plots were fertilized with 300 lbs. of N as urea annually both years. Plots were harvested six times annually for two years with a Swift Current forage harvester (Swift Current, Saskatchewan). All Individual yield measurements were recorded and samples analyzed for forage quality at Dairy One lab, Ithaca, NY. A forage value index was developed to rank individual varieties and estimate milk production potential and beef gain potential per acre.

Results

Forage ryegrass plots were grown, harvested and analyzed six times a year over a two year period of time. Table 1 and table 2 show a listing of all twenty varieties in the study and dry matter production for each cultivar over the study period. The last column on each table is the cumulative dry matter production for each cultivar over all six cuttings through the growing season for each year. In 2015, the variety Trojan had the highest dry matter yield all of twenty varieties tested at 15,085 lbs. DM per care (Table 1). That same year the lowest production variety was Oroverde at 12,503 lbs. DM per acre. Table 2 shows DM produced for each of the twenty cultivars by cutting in 2016. This year the top DM producer was Alto AR37 at 17,393 and the lowest in year two was Albion at 14,340 lbs. DM per acre.

Energy from pasture is the result the digestion of soluble and structural carbohydrates, proteins, starch and fats. Net energy for lactation (NEl) is the estimated feed energy available for maintenance and milk production after digestive and metabolic losses. Figure 1 illustrates all 20 varieties studied in 2015 and their approximate forage value for milk production per acre. Figure 2 is a bar graph illustrating the range in estimated total energy observed for the second year of the study. Not surprisingly many varieties that were at the top of the graph for year one were there again for year 2.

A cow's total energy requirement will be the sum of what she needs for each function, maintenance, growth and production. For example, a cow weighing 1300 pounds (590 kg) making 100 pounds (45.5 kg) of milk containing 3.5% milkfat will require 9.57 Mcal/day for maintenance and 31 Mcal/day for milk production. That makes her total NEl requirement equal to 40.57 Mcal/d or 0.78 Mcal/pound of dry matter if she consumed 52 pounds of dry matter per day (Ondarza). With these assumptions, we can estimate how many Mcals per acre we have grown by a variety and estimate the cwt potentially produced per acre. With a similar approach, we can estimate the energy needs for gains on a steer and figure out approximately how many pounds of beef could be gained if we had a known quality of energy. Table 3 shows the forage value index for both growing seasons combined, the relative value compared to the others in the study, and estimated number of cwt of milk per acre and an estimated lbs. beef gained for an intensive grazing beef operation.

Conclusions

Ryegrass variety trails looking specifically looking at expected animal performance are limited in the US. Chapman (2016) has clearly indicated in New Zealand the challenges with environment x genotype interactions that may change the way one cultivar performs in a specific environment compared to others. This ryegrass performance data has clearly shown many ryegrass cultivars from around the world perform well on the Oregon coast, however differences in performance were identified.

Many approaches could have been used to develop an index for milk production but energy is almost always the limiting factor in determining milk production performance of ryegrass. Accounting for energy derived from the digestion of fats, proteins, soluble carbohydrates, starches and structural fiber is the most reasonable way to estimate performance per acre of these cultivars and is what I have expressed in Table 3. Estimated milk production per acre ranged from 284 cwt. to 241 cwt. for the varieties tested. This 43 cwt difference or range could be estimated to be worth from $600-800 dollars and acre difference in total milk produced potentially (for milk valued at $15.00-20 per cwt.). This difference is seen between these advanced genetics our seed companies have been developing, selecting and breeding. Imagine how much variation you might see between these advanced genetics and unimproved pastures. This study not only identifies major performance differences between modern cultivars but it also has to make one wonder how older varieties would compare.

Shown in Table 3, the potential gains per acre for a 750 lb. steer gaining 3 lbs. a day was also estimated. Our top three varieties were estimated to produce over 1000 lbs. of gain per acre per year and the range from the highest to the lowest was over 150 lbs.

This work clearly demonstrates the production potential for using the modern cultivars in your grazing and silage systems. Genetic differences occur and I think this further supports the idea as livestock operators we should be using modern forage genetics for superior performance and productivity and we should continually monitor all our pastures/paddocks so we understand how productive they really are. You cannot successfully manage something you do not measure.

Acknowledgements

This study was financially supported by the Oregon Beef Council.

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Table 1. Dry matter yields per cutting for six cuttings and total dry matter yield for 2015.

	1	2	3	4	5	6	Total
Trojan	2544	1551	2660	2827	2891	2613	15085
TrojanNEA2	2301	1977	2590	2803	2920	2194	14786
Aberzest	2334	1851	2641	2424	2617	2567	14434
Alto	2687	1623	2397	2526	2706	2418	14357
Aberstar	2328	1727	1972	2860	2498	2931	14316
Elgon	2664	2014	2403	2636	2373	1986	14076
Remington	1880	1793	2245	2572	2615	2293	13964
Dromoro	2142	1770	1976	2350	3131	2580	13950
BealeyNEA2	2208	1500	2461	2588	2840	2308	13906
Dunlace	2648	1785	2160	2581	2268	2350	13792
Albion	2573	1759	2186	2302	2752	2130	13702
Bealey	2230	1564	2245	2572	2615	2293	13519
Drumbo	2232	1490	2432	2252	2928	2148	13482
Polim	2245	1934	2281	2451	2463	2068	13441
Calibra	2428	1551	2523	2469	2175	2179	13326
Tyrella	2134	1621	2542	2162	2997	2001	13304
AltoAR37	2117	1621	2223	2377	2534	2115	12988
Kentaur	2282	1384	2285	2611	2254	2060	12876
Tetragain	2147	1274	2287	2728	2207	1982	12625
Oroverde	2335	1314	2455	2233	2574	1592	12503

Figure 1. Perennial ryegrasses rank by milk production index for 2015.

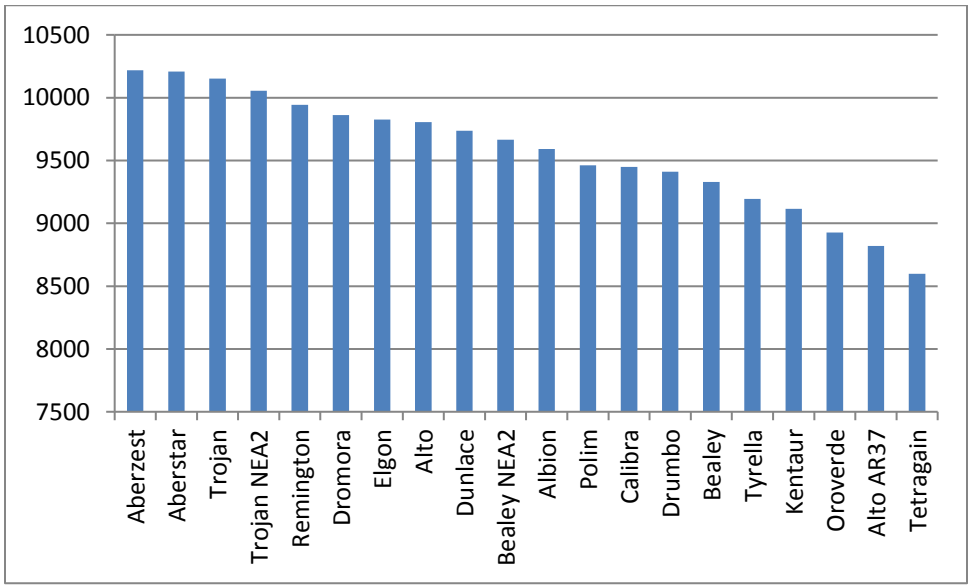

Table 2. Dry matter yields per cutting for six cuttings and total dry matter yield for 2016.

	1	2	3	4	5	6	Total
Alto AR37	3323	2767	3088	3354	2764	2097	17393
Aberzest	3148	3344	3078	3105	2385	2176	17237
Trojan	3484	2368	3619	2475	2901	2296	17143
Alto	3252	2911	3363	3181	2490	1916	17113
Aberstar	3186	2453	3103	3508	2697	2128	17075
TrojanNEA2	3068	2726	2909	3305	2737	1966	16711
BealeyNEA2	3318	3012	3479	2674	2378	1998	16859
Calibra	3010	3283	3534	2278	2031	1897	16032
Elgon	3170	2746	3163	2556	2437	1926	15998
Remington	2688	3036	2997	2758	2443	2049	15970
Dromoro	2971	2801	2966	2806	2612	1812	15969
Bealey	3096	2635	3202	2574	2330	1830	15667
Polim	2998	2632	2977	2698	2188	1906	15399
Kentaur	3417	3142	2975	2303	1642	1857	15337
Drumbo	2895	2959	2504	2773	1976	1937	15045
Tetragain	3380	2788	3015	1863	2183	1684	14913
Dunlace	2539	2505	3282	2335	2699	1618	14978
Oroverde	3224	2944	2450	2431	2055	1608	14712
Tyrella	2579	2608	2466	2550	2177	2295	14677
Albion	3436	2291	2318	2282	2234	1779	14340

Figure 2. Perennial ryegrasses rank by milk production index for 2016.

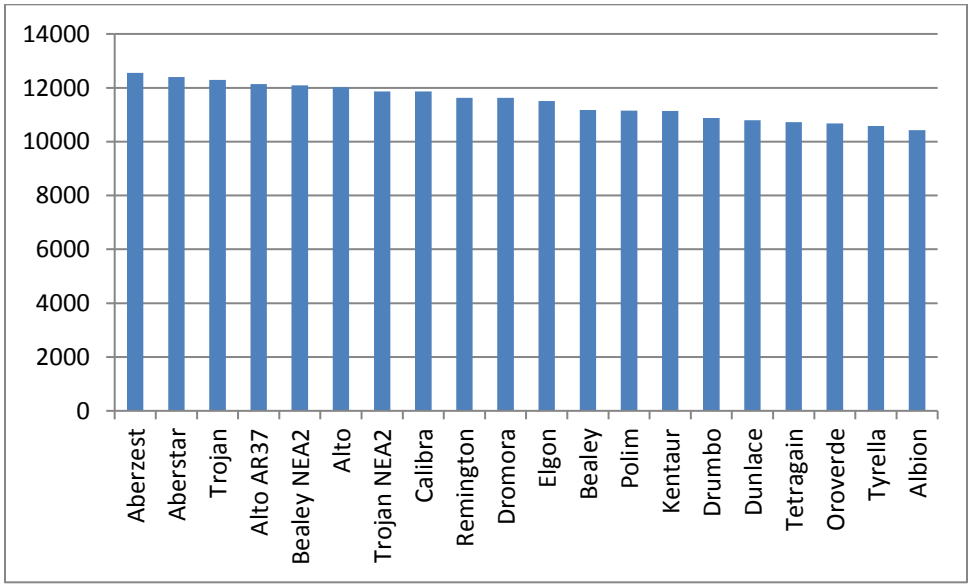

Table 3. Perennial ryegrasses ranked for milk production index, relative value, cwt milk per acre, lbs. gain potential per acre.

	Index Value	Relative Value	Milk CWTs/Acre*	Lbs. Gain/Acre**
Aberzest	11384	108	284	1034
Aberstar	11301	107	282	1027
Trojan	11221	106	280	1020
TrojanNEA2	10959	104	274	996
Alto	10909	104	272	991
BealeyNEA2	10876	103	272	989
Remington	10784	103	270	980
Dromoro	10743	102	269	977
Elgon	10663	101	266	969
Calibra	10655	101	266	968
Alto AR37	10480	100	262	952
Polim	10305	98	258	936
Dunlace	10268	97	257	933
Bealey	10249	97	256	931
Drumbo	10143	96	253	922
Kentaur	10125	96	253	920
Albion	10008	95	250	909
Tyrella	9887	94	247	899
Oroverde	9803	93	245	891
Tetragain	9660	92	241	878

* Milk production estimates based off the assumption of a Holstein cow producing 100 lbs/day (40 Mcal/day).
** Weight gain estimates based off a 750 lb steer gaining 3 lbs a day (11 mCal/day).

Oregon State University

BEEF148

Beef Cattle Sciences

Oregon Beef Council Report

Research on Stream Water Temperature in Riparian Systems[1]

Carlos G Ochoa[2]

Synopsis

Ongoing research related to stream temperature, groundwater, and vegetation interactions in riparian areas.

Summary

The objectives of this ongoing research effort are: 1) to better understand how stream water temperature is affected by the interactions between surface water and shallow groundwater, and; 2) to determine the overall effects that land and vegetation management practices have in stream water temperature. The project is being conducted in the fifteen-mile creek watershed in Wasco County. Multiple riparian areas in several creeks within this watershed have been under CREP for a good number of years, allowing mature riparian vegetation to establish. Beginning in the summer of 2014, we instrumented several locations along the creek to measure different water and weather variables. Also, groundwater and vegetation information were collected. We evaluated changes in stream temperature dynamics as affected by vegetation structure, streamflow, and stream-aquifer interactions in a 1-km reach along the creek. We determined vegetation structure and estimated canopy cover along the riparian area. We installed multiple stream, groundwater, and ambient temperature sensors at selected locations along the reach. Stream temperature sensors were installed at shaded and exposed locations. In the summer of 2015, we used Distributed Temperature Sensing (DTS) and fiber optic technology to accurately measure stream water temperature at fine temporal (every 15 min) and spatial (every one meter) resolution scales. DTS data findings were compared to data obtained from point specific temperature sensors (17) installed in the creek. Preliminary results show Gray Alder (Alnus incana) and Reed Canary Grass (Phalaris arundinacea) were the dominant overstory and understory species. No significant changes in stream temperature were observed in shaded versus exposed sensor locations. Greater stream temperature levels were observed between 3 and 6 pm in most days and stream temperature fluctuations followed those from ambient temperature. A close agreement between stand-alone sensor and DTS stream temperature measurements was observed. The finer spatial resolution of the fiber optic cable measurements allowed for a better understanding of vegetation-stream temperature dynamics. Surface and subsurface flow contributions from a tributary meeting the creek resulted in minor changes in stream temperature conditions at the confluence.

Introduction

Accounting for non-point source pollution is of concern when trying to implement best management practices aimed to improve water quality conditions. Different agriculture practices have been targeted as sources of thermal and sediment pollution. For example, overland surface runoff from non-

controlled irrigation carries water that is typically warmer than water in the stream and that can be considered a source of thermal pollution. A reduction in stream flows associated with increased stream water temperature can be detrimental for important fish species such as Coho salmon, Chinook salmon, and steelhead among others. Increases in river water temperature are inversely related to dissolved oxygen levels and that can severely affect fish species survival.

Having a better understanding of the thermal environment in several locations along the stream can help decision makers when assessing programs aimed to meet temperature reduction objectives (Larson and Larson, 2001). One of the factors that can affect the thermal environment of the stream is the confluence of groundwater with the stream. Groundwater temperature can be lower than stream water temperature, particularly during summer time and low flow conditions when surface water is more affected by direct sunlight heating. Under certain conditions, contributions of groundwater flow to the stream flow may help reducing the temperature of the water in the stream (Stringham et al. 1998). However, this will be heavily dependent on the amount and characteristics of the groundwater reaching the stream.

Upland water sources and lower valleys can be hydrologically connected through surface water and shallow groundwater interactions, particularly in the forms of precipitation, runoff and infiltration in the upper watershed and their linkages with streamflow, irrigation, and aquifer recharge in the lower valleys (Ochoa et al 2013). Proper understanding of surface water and groundwater interactions and of how these interactions can be impacted through agriculture related practices becomes critical to properly address concerns related to water quality. The objectives of this three-year ongoing research effort are: 1) to better understand how stream water temperature is affected by the interactions between surface water and shallow groundwater, and; 2) to determine the overall effects that land and vegetation management practices have in stream water temperature and runoff/sediment generation processes.

Materials and Methods

In July 2014, we installed four monitoring stations along the fifteen-mile creek, and two along the eight-mile creek. Additionally, we installed sensors to measure ambient relative humidity at two selected locations in the eight-mile creek. A full weather station and two monitoring wells were installed in one of the locations along the fifteen-mile creek. All sensors were programmed to record variables in time intervals ranging from 15 min (weather station) to 1 hour (temperature and groundwater sensors). Significant stream temperature differences observed in a valley location motivated to begin intensively monitoring of this area to determine potential vegetation-stream-aquifer interactions that may be affecting the water temperature in the stream. We evaluated changes in stream temperature dynamics as affected by vegetation structure, streamflow, and stream-aquifer interactions in a 1-km reach along the creek. We determined vegetation structure and estimated canopy cover along the riparian area. We installed multiple stream, groundwater, and ambient temperature sensors at selected locations along the reach (Figure 1). Stream temperature sensors were installed at shaded and exposed locations. In the summer of 2015, we used Distributed Temperature Sensing (DTS) and fiber optic technology to accurately measure stream water temperature at fine temporal (every 15 min) and spatial (every one meter) resolution scales. DTS data findings were compared to data obtained from point specific temperature sensors (17) installed in the creek.

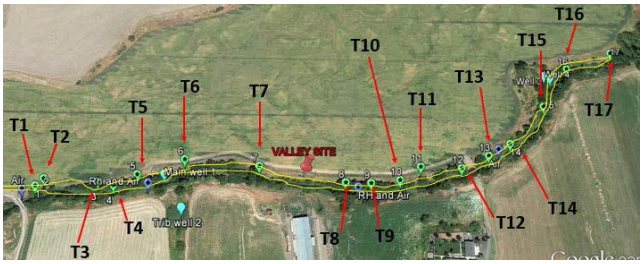

Figure 1. Map of the 1-km reach along the fifteen-mile creek illustrating monitoring well (light blue), stream temperature (green), and DTS fiber optic cable (yellow line) installed.

Results

Study results showed higher stream temperature in the downstream valley locations when compared to two upstream locations. Stream temperature in upstream locations ranged from 40 to 68 degrees Fahrenheit, while in the valley location was consistently higher with values ranging from 44 to 82 degrees Fahrenheit (Fig.2). Ambient temperature was similar in upstream and valley locations, with values ranging from 42 to 104 degrees Fahrenheit in the monitored summer time. No significant difference in stream water temperature was found

across sensors, irrespective if they were located in shaded or non-shaded areas.

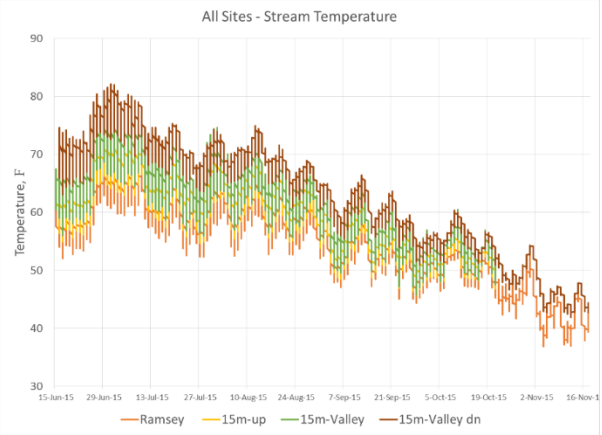

Figure 2. Stream water temperature fluctuations in upstream creek (15m-up), upstream tributary (Ramsey), valley near Dufur (15m-Valley), and downstream (15m-Valley dn) locations along the fifteen-mile creek during Summer-Fall of 2015.

Figure 3 illustrates higher stream temperature fluctuations in a downstream valley location when compared to an upstream location for a one full year including two summer seasons. Greater stream temperature differences were observed during the summer months. At both, upstream and downstream, locations stream temperature reached near freezing conditions several times during the winter months.

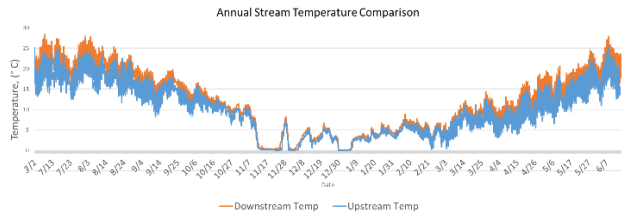

Figure 3. Stream water temperature fluctuations in upstream creek and downstream locations along the fifteen-mile creek during Summer 2014 to Summer 2015.

Data derived from the DTS equipment showed highest stream temperature (~ 71 F) occurred between 3 and 6 pm in most days during the summer season. Coolest temperatures were observed in early morning at around 6 am (Fig. 4). DTS-measured stream temperature fluctuations followed those from ambient temperature, and a close agreement between stand-alone sensor and DTS stream temperature measurements was observed.

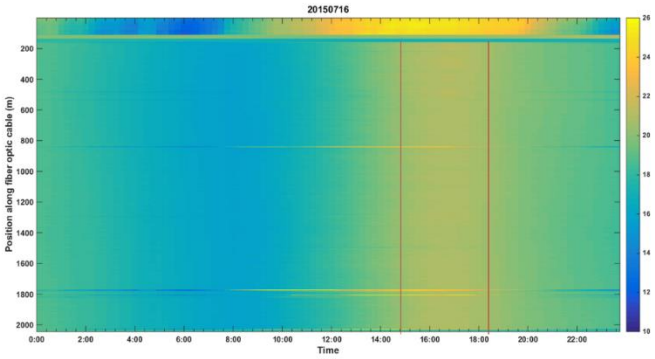

Figure 4. Stream water temperature (Celsius) along a 2,000 m cable located in the valley, along a 1-km reach of the fifteen-mile creek during mid-July to end of September of 2015.

Results from this ongoing experiment indicate that water temperature fluctuations in the stream, particularly in the valley, may be subject to other factors other than that those provided by vegetation shading. For example, figure 5 shows that increases in stream flow during the snow-melt runoff season in January 2016 significantly reduced subsurface flow temperature in a seasonal tributary to the fifteen-mile creek at the valley site. Stream temperature in the creek may have decreased due to higher streamflow levels in the creek but also to subsurface flow contributions.

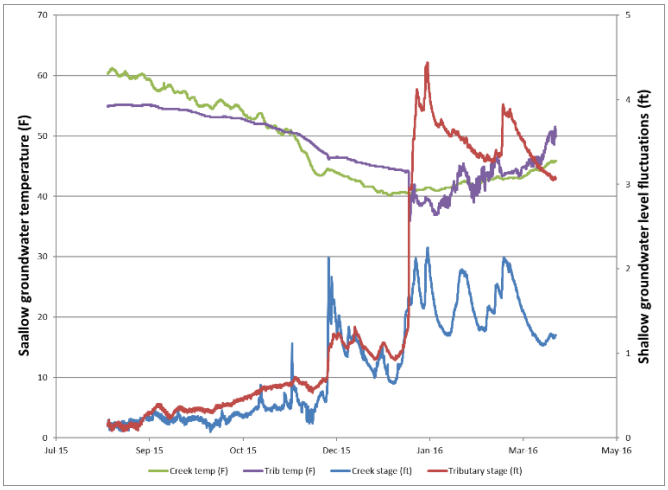

Figure 5. Shallow groundwater level and temperature fluctuations at the valley site.

Riparian vegetation water uptake, specifically from dominant overstory vegetation (gray alder) has been monitored at the valley site using sap flow sensors. Figure 6 shows that a mid-size (around 7 m tall) gray alder tree can use up to 50 gallons of water a day. During the period of record 13 July through 7

August 2015, peak transpiration rates ranged between 15 and 50 gal/day.

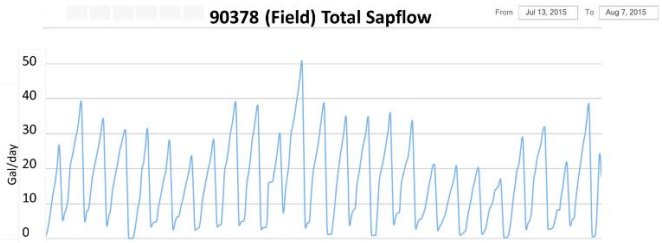

Figure 6. Water uptake by a mid-size gray alder tree during the summer of 2015.

Conclusions

Results from this ongoing data collection effort indicate that multiple factors may intervene in regulating stream temperature. Vegetation shading does not seem to be the only factor that regulates stream temperature along the different areas monitored in this semiarid riparian system. Therefore, a systems-based, or holistic, approach is recommended when evaluating the potential effects that land management practices may have on water quality parameters (for example, stream temperature) along arid and semiarid riparian areas.

Acknowledgements

This research study is financially supported by the Oregon Beef Council. Author wishes to acknowledge the support provided in conducting this research effort by the Water Committee of the Oregon Cattlemen's Association, by Keith Nantz (Dillon and Cattle Co.), and by the Fifteen-mile Watershed Council. Also, thanks to local producers in Wasco County for granting access to their properties for installing monitoring devices.

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Beef Cattle Sciences

Oregon Beef Council Report

Progress Reports – Rangeland Ecology and Management[1]

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

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Project Objectives: The objective of this study is to determine which seeding techniques are appropriate for enhancing the establishment of forage kochia; a promising restoration plant for improving the diversity, productivity, and later-season forage quality of degraded semi-arid sagebrush rangelands. Specifically, we will evaluate two seeding methods, two seasons of seeding, two seed treatments and the efficacy of stored versus freshly harvested seed to determine which combination of seeding techniques produces the best results.

Project Start Date: Summer of 2014
Expected Project Completion Date: Fall of 2017

Project Status: Three study sites were selected and exclosures were erected around each site during the summer of 2014. Also in 2014, existing vegetation within the exclosures was treated with glyphosate during the summer and then burned with a trailer mounted propane torch in the fall to prepare a seedbed for seeding treatments. The following seeding treatments were applied to 6 X 18 ft plots arranged in a randomized block design, with 5 replicated blocks per study site:

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

Year 1 fall and winter seeding treatments were conducted during early November 2014 and early February 2015, respectively. Year 2 fall and winter seeding treatments were conducted during late October 2015 and early February 2016, respectively. Forage kochia was seeded at 2 lbs/ac pure live seed in all plots that received a seeding treatment. Vegetation cover and density by species were measured during the summers of 2015 and 2016 in each treatment. Year 1 data were summarized and preliminary results are presented and discussed below. Year 2 data have been entered and are currently being quality checked and summarized.

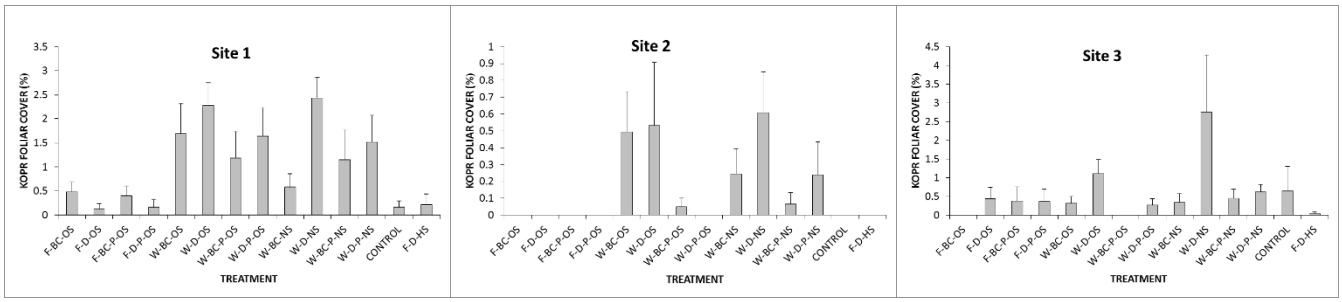

Figure 1. Cover (mean ± SE) of forage kochia the growing season after Year 1 seeding treatments were applied. F-BC-OS: Fall broadcast of year-old seed; F-D-OS: Fall drill of year-old seed; F-BC-P-OS: Fall broadcast of pillows made with year-old seed; F-D-P-OS: Fall drill of pellets made with year-old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

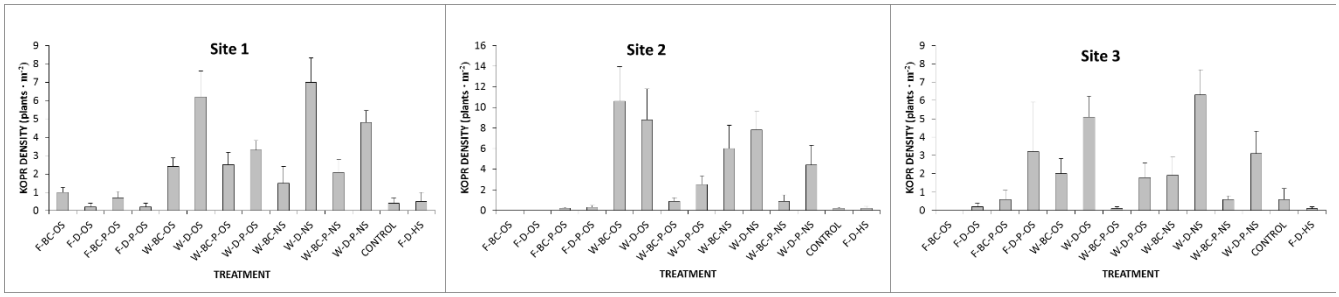

Figure 2. Density (mean ± SE) of forage kochia the growing season after Year 1 seeding treatments were applied. F-BC-OS: Fall broadcast of year-old seed; F-D-OS: Fall drill of year-old seed; F-BC-P-OS: Fall broadcast of pillows made with year-old seed; F-D-P-OS: Fall drill of pellets made with year-old seed; W-BC-OS: Winter broadcast of year-old seed; W-D-OS: Winter drill of year-old seed; W-BC-P-OS: Winter broadcast of pillows made with year-old seed; W-D-P-OS: Winter drill of pellets made with year-old seed; W-BC-NS: Winter broadcast of new seed; W-D-NS: Winter drill of new seed; W-BC-P-NS: Winter broadcast of pillows made with new seed; W-D-P-NS: Winter drill of pellets made with new seed; CONTROL: Not seeded; and F-D-HS: Fall drill of old seed with hydrophobic coating.

Preliminary Results and Discussion: Preliminary study results from year 1 of the study suggest seeding during the winter may be more effective for establishing forage kochia than fall seeding, regardless of whether stored (i.e., year-old) or freshly harvested seed is used (Figures 1 and 2). Treatments will be repeated during the fall and winter of 2015 and 2016, respectively, to confirm this result, but it appears that fall-seeded forage kochia might germinate and emerge shortly after planting and subsequently suffer high winter mortality, whereas emergence of seed sown in the winter is likely delayed until a more favorable time during the spring. In addition, preliminary results indicated shallow drill seeding conducted in the winter may have produced better results than broadcasting seed; at least on two of three sites (Figures 1 and 2). Preliminary results also suggest bare seed, either broadcasted or drilled, performed as well if not better than seed sown in pillows or pellets, enhancements designed to improve seed germination and emergence environment, suggesting seed germination and emergence may not be major limiting factors for forage kochia establishment. Similarly, seeds that had been stored for a year and then treated with a hydrophobic coating, an enhancement designed to delay germination of fall-planted seed until spring, performed poorly compared to similar seed (i.e., year-old seed) planted in the winter. Again, preliminary results of this study indicate winter seeding is superior to fall seeding which suggests delaying germination of fall-seeded forage kochia until late winter or spring should improve establishment performance of

forage kochia. Therefore, we suspect the amount and/or formulation of the hydrophobic coating tested in this study needs adjustment, and deserves further investigation.

Future Plans: Final vegetation sampling will be conducted during the summer 2017. Analysis of variance (ANOVA) will be used to determine treatment effects. Results will be published in a final report to the Oregon Beef Council and a scientific journal during the fall of 2017 and winter of 2018, respectively. The research team conducting this project genuinely appreciates the support of the Oregon Beef Council.

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

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Project Objectives: The project research objective is to study annual warm season grasses production and quality under different irrigation scenarios. The specific objectives are:

1) Screen annual warm season grass forage **species and cultivars** for forage species and cultivars selection;
2) Compare annual warm season grasses forage production and quality under different **water scenarios** in eastern Oregon.

Project Start Date: July of 2016
Expected Project Completion Date: December of 2018

Project Status: We seeded five annual warm season grasses, including proso millet, foxtail millet, teff, pearl millet, and soghum-sudan hybrids, along with five species of annual cool season grasses, annual legumes, and annual brassicas July, 2016. Irrigation in 2016 was carried out same for all the irrigation scenarios due to the time required for testing our new irrigation system. The test plots were fertilized, weeded, and monitored from July to August of 2016. We harvested the plots in the first week of September right after the first noticeable frost. The preliminary data was shown in figure 1 and 2. In summary, annual cool season grasses produced higher than annual warm season grasses (Figure 1). Among annual warm season grasses, short growing season species proso millet produced the most (Figure 2).

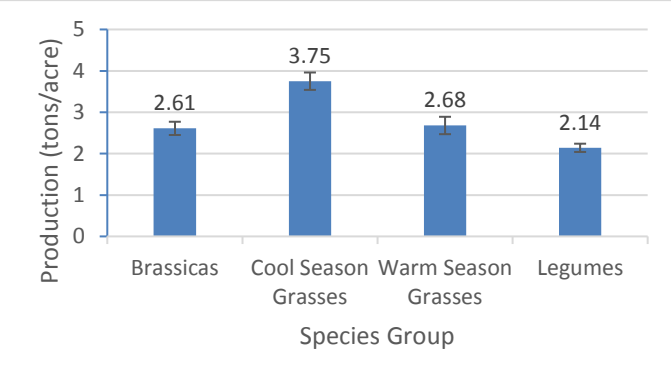

Figure 1. Forage production for each group including annual warm season grasses.

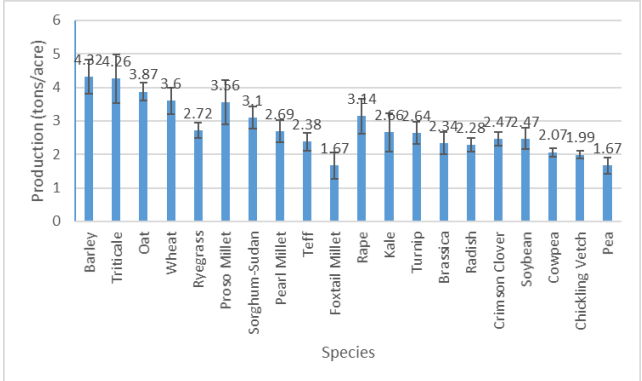

Figure 2. Forage production for each species including five annual warm season grass species. The first five species are annual cool season grasses, the second five species are annual warm season grasses, the third five species are annual brassicas, and the last five species are annual legumes.

In the coming year of 2017, we will apply the proposed irrigation treatment to the field test plots. We will seed the annual forages according to the weather. Production data will be collected. Furthermore, forage quality lab analysis will be carried out. This study will be continued into 2018 growing season due to the 2016 growing season irrigation treatment preparation.

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

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

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

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

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

Project Status: We have hired an undergraduate student majoring in Rangeland Sciences to assist this fall with the collation of data and mapping using ArcGIS. Field data collection does not start until spring and summer of 2017. In the meantime, we are also working on a summary article regarding this research for the journal Rangelands and leveraging this grant for additional funding through the USDA. Data analysis and a write-up of the final report could be completed by October 2017.

Preventing juniper reestablishment into sagebrush communities: Improving the watershed function

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Project Objectives: 1) To characterize the progression of juniper re-occupation of sagebrush communities ten-years after tree removal, and; 2) To evaluate potential impacts of preventing juniper re-occupation on sagebrush steppe vegetation and hydrologic dynamics using prescribed fire as a secondary treatment.

Project Start Date: N/A
Expected Project Completion Date: 2019

Project Status: We are in the process of analyzing historic vegetation and hydrologic data collected at the study site. Additionally, we have added two monitoring stations to quantify effective precipitation as affected by juniper canopy interception. Also, we recently began vegetation monitoring to assess the number of juniper saplings in

the treated area. Results from an exploratory phase indicate a sapling density of 45 trees/acre, which is similar to tree density counts for mature trees in the untreated watershed. In addition, we are installing new soil moisture stations to document precipitation-interception-soil moisture dynamics in treated and untreated areas. Research activities are following their expected course, funding for this project has just started. This project will add critical information regarding how quickly juniper moves back into a treated site. Also, new data collected will allow us to enhance understanding of sagebrush steppe vegetation and water relationships in treated versus non-treated sites.

Research on Stream Water Temperature in Riparian Systems

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Project Objectives: 1) To better understand how stream water temperature is affected by the interactions between surface water and shallow groundwater, and; 2) To determine the overall effects that land and vegetation management practices have in stream water temperature.

Project Start Date: N/A
Expected Project Completion Date: 2017

Project Status: Currently, we are monitoring stream and ambient temperature at 4 different locations along the 15-mile creek in Wasco County. We are in the process of analyzing data from an intensive data collection effort that occurred in one location in the valley, where a fiber optic cable was used to accurately measure stream temperature. Additional sensors (20) were installed to take independent measurements of stream and ambient temperature. Four monitoring wells installed provide important data regarding shallow groundwater level and temperature fluctuations. A weather station was also installed at this site. Riparian vegetation water uptake that may affect both surface water and groundwater is also being calculated based on collection of weather and tree stem-flow data, hemispherical view images, and from a riparian vegetation assessment effort.

Research activities are following their expected course; additional resources are being brought in to strengthen the project. A MS student in Water Resources Science is fully dedicated to this project, a PhD student in Rangeland Ecology and Management is also assisting with data analysis and manuscript preparation.

Expected results will provide sound scientific understanding of some of the aspects that affect water quality in semiarid riparian areas and the need for using a systems-based approach when trying to implement best management practices aimed to reduce non-point source pollution and improve water quality conditions.

South warner sage-grouse response to juniper removal

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Project Objectives: The overall objective of the OSU study is to build upon the existing database and provide a longer term assessment of the effects of juniper removal on sage-grouse habitat use and demography. The specific objectives during this phase of the project are 1) modeling sage-grouse habitat selection during nesting and brood rearing before and after juniper treatments, 2) modeling the demographic and population response of sage-grouse to juniper management, and 3) modeling landscape resistance to sage-grouse movements in relation to juniper. Additionally, we are transitioning from VHF radio collars to GPS PTT backpacks.

Project Start Date: 2015
Expected Project Completion Date: 2017

Project Status: The BLM completed the remaining 1,949 hectares of juniper treatments in 2016, resulting in a total of 10,249 hectares treated between 2011 and 2016. The treatments included hand cutting, piling, burning, and limited machine removal. Additionally, the Natural Resources Conservation Service treated 2,997 hectares on private lands in the project area between 2010 and 2014.

FIELD WORK : Winter weather prevented trapping during February 2016. However, during March and April of 2016, we captured 37 sage-grouse hens in the treatment and control areas which brought our sample size at the beginning of the field season in the treatment to >40. Inclement weather and impassible road conditions prevented trapping in the control in March in addition to February, resulting in a sample size at the start of the field season that was <40. We recorded 511 individual locations of VHF marked hens and 35,140 locations of GPS marked hens over the course of the field season. We located 69 nests including 13 second nests and one third nest. Vegetation plots were completed at all 69 nests and at 69 paired random points.

OBSERVATIONS: Snow fall during the winter of 2015/2016 was much closer to normal than the previous winter. The subsequent spring runoff filled the reservoirs and stock tanks across the study area and created significantly more summer and late brood rearing habitats than the previous summer. As a result, marked sage-grouse seemed to be more dispersed across the project area during the summer months, particularly in the treatment. Most of the sage-grouse in the control still made summer migrations to high elevations in Nevada or low elevation meadows and irrigated fields in California.

Brood survival was low this year in both the control and treatment despite favorable habitat conditions (Table 1). This was likely due to the May 20 snow storm that blanketed the study area with heavy, wet snow. At least 14 of the 25 successful nests in the project area hatched prior to May 20 and most had very young (<2 weeks) broods. Peak hatch periods for first nests occurred during the weeks of May 1 (n=10) and May 15 (n=8). Peak hatch for second nests was the first week of June (n=2). Other sage-grouse research projects that experienced the same weather event also observed reduced brood survival (Christopher Anthony and Phillip Street, personal communications).

FUTURE WORK: Due to the difficulties capturing sage-grouse during the late winter and early spring, particularly in the control, we plan to focus our efforts for the 2017 field season during the fall when road conditions should be favorable. The BLM purchased 25 additional GPS PTT's which will arrive prior to fall trapping.

I hope to begin data analysis and writing during the 2017 field season. In order to do this, we may hire an additional field technician so that I do not have to spend as much time in the field as I have during the last two field seasons.

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

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

Project Start Date*:* Fall 2016

Expected Project Completion Date: Fall, 2019

Project Status: Preliminary alfalfa and weed yield has been compiled for both organic alfalfa fertility trials (18 and 12 treatment comparisons) that were conducted on the Dan Bansen Farm, Fort Rock, Oregon. Trial I (2012-2015) and Trial II (2013-2015) field work has been completed. Cost of these treatments ranged from $0/acre to

$450/acre. Presently plant samples and soil samples are being ground and being readied for lab analysis in 2017. We are hoping that we can run the samples through the new and updated Plant and Soil Analytical Lab at OSU. (Originally we were going to run the soil samples with AgSource at Umatilla, OR and the plant samples at Brookside Lab, OH). The final trial year (2015) soil sample testing is being funded by Oregon Dairy Farmers Association and the final year trial plant sample nutrient testing is being funded by Oregon Beef Council. All replications will be tested for the last year will be tested to determine definitive statistical difference between treatments or lack thereof. Previous years soil and plant sample replications will be pooled for testing to determine trends. More grants will be sought based on the 2015 testing results, to continue working our way through testing the previous year's samples (2012-2014).

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

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

REPORT STATUS OF STUDIES FUNDED BY THE OREGON BEEF COUNCIL

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

Projects funded in 2007 – 2008 FY

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

Projects funded in 2008 – 2009 FY

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

Projects funded in 2009 – 2010 FY

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

Projects funded in 2010 – 2011 FY

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

Projects funded in 2011 – 2012 FY

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

Projects funded in 2012 – 2013 FY

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

Projects funded in 2013 – 2014 FY

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

Projects funded in 2014 – 2015 FY

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

Projects funded in 2015 – 2016 FY

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

Projects funded in 2016 – 2017 FY

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

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Beef Cattle Sciences

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