AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
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TITLE: Does Feeding Selenium-Enriched Alfalfa Hay For Eight Weeks Improve Performance And Health Of Weaned Beef Calves?

RESEARCH LEADER:
Jean A. Hall, DVM, PhD, Dipl. ACVIM (Internal Medicine), Professor, Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University; 24 yr at OSU; 541-737-6537 (office); Jean.Hall@oregonstate.edu

COOPERATORS:
Gene J. Pirelli, M.S., Professor, Department of Animal Sciences, Polk County Extension, 289 E. Ellendale, Suite 301, Dallas, OR 97338; 36 years at OSU; (503) 623-8395 (office); Gene.Pirelli@oregonstate.edu

SUMMARY:
Our overall goal is to improve production and health of beef cattle in Oregon by increasing the practice of selenium (Se) fertilization, also known as agronomic biofortification. Major parts of the US are deficient in Se. Regional differences are reflected in Se content of forages and in animals consuming them. Selenium is incorporated into selenoproteins, whose functions range from antioxidant, anti-inflammatory, and detoxification to thyroid hormone activation. We are actively working on all aspects of agronomic biofortification with Se, in order to provide data that shows cattle fed Se-enriched forage have improved production performance and improved immune responses resulting in improved health, and that feeding Se-enriched forage to cattle improves feedlot performance. For this proposal, we plan to demonstrate that feeding Se-biofortified forage for 8 weeks to weaned beef calves during a backgrounding program improves production efficiency (measured as improved weight gain). We believe that supranutritional Se supplementation has growth promoting properties independent of the antioxidant properties of selenoproteins and their effects on the immune response. This likely occurs directly, or indirectly, through regulation of transcription factors involved in cell growth and control of apoptosis as well as maintenance of cellular redox status, e.g., via iodothyronine deiodinases (responsible for metabolism of thyroid hormones) and thioredoxin reductases (reduce thioredoxin). In addition, we will assess immune responses to demonstrate that feeding Se-enriched forage optimizes immune responses in a way that optimizes health of calves before entering the feedlot. During an 8-week backgrounding program, we will monitor whole-blood Se levels, antibody titers to common vaccinations, immune cell function assays, as well as morbidity and performance.

Selenium has been recognized for years as an essential trace element for animals. The Pacific Northwest region is among those with the lowest amounts of Se in soils and plants. In the US, a survey of state veterinarians and state veterinary diagnostic labs revealed that Se-deficiency diseases were diagnosed in 46 states and were reported to be an important livestock problem in geographic regions of 37 states. In general, the majority of cattle raised in low-Se regions do not receive sufficient dietary Se for optimum health. Calves may have greater Se requirements during periods of stress. Providing adequate Se is important to prevent Se-deficiency diseases

such as nutritional myodegeneration (white muscle disease) and Se-responsive unthriftiness. Although the essentiality of Se supplementation has been known for five decades, the most effective method of Se delivery to cattle to achieve optimum performance is still being investigated. The most promising Se supplementation method is Se fertilization, as it increases Se concentrations in plants, and in animals consuming Se-biofortified forages and hay. Nitrogenous fertilizers, widely hailed as one of the most important advances in agricultural technology, increase biomass, but dilute essential minerals like Se, emphasizing the need for Se amendments. Se-fertilization has been used in several countries including Finland, Denmark, New Zealand, and the United Kingdom to increase Se concentrations in the food chain. In Oregon, the Department of Agriculture does not control the use of Se as a plant fertilizer, making it possible to produce Se-biofortified forages. We believe Se fertilization is economical and practical and can be readily adapted to Oregon hay and cattle production systems. Our goal is to provide solid data on the health benefits so that this practice becomes routine in Oregon.

Optimal immune function is critical for calves experiencing the stress of weaning, relocation to feedlots, and commingling with animals of different origins. The National Agriculture Statistics Service shows that 516,000 calves were born in Oregon in 2014. The majority of these Oregon grown calves enter the feedlot. Even with good calf-hood vaccination programs, producers often encounter significant health issues in the feedlot, including mortality. Reducing these losses by increasing immunity to feedlot diseases would have a significant economic impact for Oregon cattle producers. It is known that the transition period between weaning and movement to a feedlot is one of the most stressful times for beef calves. Performance in weaned beef calves is enhanced if a preconditioning program is utilized before calves enter the feedlot. Several weeks in a preconditioning program are recommended to reduce the stress associated with weaning, dehorning, castration, and vaccination with the goal of reducing morbidity and mortality after arrival at the feedlot. Because Se is thought to play an important role in the immune response of cattle, calves may have greater Se requirements during the backgrounding and transitional period.

OBJECTIVES:

The objective of this study is to demonstrate that feeding Se-biofortified forage enhances production, immunity and health of weaned beef calves. *We hypothesize that feeding Se-biofortified alfalfa hay to weaned beef calves will increase whole blood-Se concentrations, enhance innate and adaptive immune responses, and increase body weight gain in Se-supplemented calves compared with control calves not receiving Se-biofortified hay*.

PROCEDURES:

To make Se-enriched alfalfa forage, sodium selenate will be mixed with water and sprayed onto the soil surface of an alfalfa field after the second cutting of hay. Two application rates of selenate will be used for the Medium-Se and High-Se forage. In a previous study we showed that fertilizing with 45.0 (M-Se) or 89.9 (H-Se) g Se/ha resulted in corresponding increases in alfalfa hay Se content of 1.55 and 3.26 mg Se/kg dry matter, respectively. Hay will be harvested from the respective field plots, and then sampled for Se content. A Penn State forage sampler will be used to take 25 cores from random bales in each alfalfa hay source.

Angus and Angus-cross calves (n=45) weighing approximately 600 lb at weaning from the Oregon State University Soap Creek ranch will undergo an 8-week back grounding period before sale to a commercial feedlot. During this 8-week back grounding period, calves will be divided into 9 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 forage Se 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 forage Se): 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 forage Se): 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.

Calves will be bled at the beginning of the treatment period (day 0) and every 2 weeks for 8 weeks to collect whole blood for Se analysis. Body weight will be measured at the beginning of the treatment period (day 0) and at the end of the 8-week feeding period to determine weight gain. Health records will be maintained and evaluated to monitor health status.

We plan to investigate immune responses to multiple vaccines. We hypothesize that humoral immunity will be enhanced after short-term exposure to high-Se-fertilized forage. Calves will be vaccinated at 2, 4, and 6 weeks with J-5 *E. coli* bacterin. To assess humoral immunity, serum will be collected for antibody titers prior to immunization and at the end of the 8-week feeding period. Titers will be assessed as previously reported using an indirect ELISA procedure. We will also vaccinate calves at 2 weeks with BOVI-SHIELD GOLD ONE SHOT™. Titers will be assessed at 8 weeks to Mannheimia [Pasteurella] haemolytica type A1, and to IBR, PI3, and BRSV to see if increased titers observed in pilot studies can be replicated with more commonly used vaccines.

To assess innate immunity we will study white blood cell functions. Activated macrophages generate large amounts of nitric oxide (NO) to damage pathogens, which can be measured by a colorimetric method when culturing macrophages in the presence of activators. In contrast to activated macrophages, regulatory macrophages convert arginine, the precursor for NO production, to urea thereby preventing NO release. Urea is also measured by a colorimetric method. We will compare the ratio of NO to urea production in macrophages isolated from blood of calves fed Se-enriched forages vs control hay to determine if Se supplementation induces a shift towards NO production, which is indicative of activated macrophages and an enhanced innate immune response. Complement proteins present in blood can directly kill bacteria, which will be measured by isolating plasma from calves and mixing serial dilutions of the plasma with laboratory strains of *E. coli* bacteria. Bacterial outgrowth will be monitored by counting colonies 24 h after plating bacteria. We will compare complement-mediated bacterial killing in plasma of calves fed Se-enriched forages vs control hay to determine if Se supplementation enhances the innate immune responses associated with complement-mediated bacterial killing. Macrophages and neutrophils are the cells primarily responsible for carrying out innate immune effector functions at sites of infection via phagocytosis, which we will measure by incubating white blood cells with fluorescently labeled latex beads and measuring bead uptake by flow cytometry.

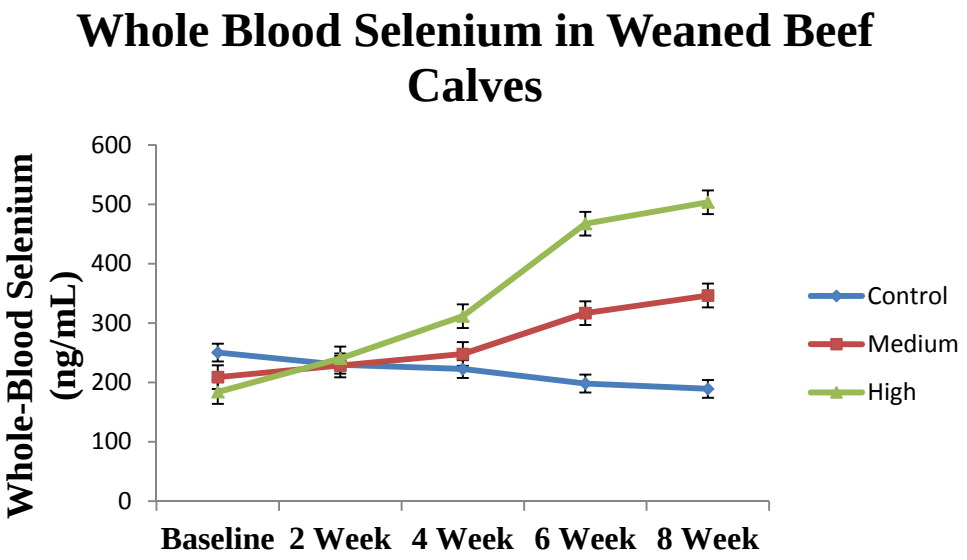
Correlating the expression levels of Se-responsive genes, as previously described by us with the functional measures of phagocytosis will allow us to address our hypothesis that Se enhances innate immune functions.

Statistical analyses: The number of pens assigned to each group (n=3) was chosen based on our previous beef calf feeding trial (Hall et al., 2013a), whereby we calculated significance in whole-blood Se concentrations at the $P < 0.01$ level between groups. In the current study, data will be evaluated after testing for normality, and evaluated using an ANOVA method for repeated measures (PROC MIXED) in Statistical Analysis Software [SAS]. Data will be reported as least square means ± SEM. Significance will be accepted at $P \leq 0.05$.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

We have submitted one paper to PLOS ONE (currently under review) looking at the effects of feeding Se-biofortified hay on the nasal microbiota of weaned beef calves. Recent evidence suggests that cattle diagnosed with bovine respiratory disease complex have significantly less bacterial diversity. The objective of this study was to determine whether feeding weaned beef calves Se-enriched alfalfa hay for 9 weeks in a preconditioning program prior to entering the feedlot alters nasal microbiota. Nasal swabs were collected during week 9 from one or two calves from each pen (total n=16). Calculated Se intake from dietary sources was 3.0, 15.6, and 32.2 mg Se/head/day for calves consuming alfalfa hay with Se concentrations of 0.34 to 2.42 and 5.17 mg Se/kg dry matter, respectively. Whole- blood Se concentrations after 8 weeks of feeding Se-fertilized alfalfa hay were dependent upon Se-application rates (0, 45.0, or 89.9 g Se/ha) and were 155, 345, and 504 ng/mL ($P_{Linear} < 0.0001$; See Figure below). Microbial DNA was extracted from nasal swabs and amplified and sequenced. Alpha rarefaction curves comparing the species richness (observed OTUs) and overall diversity (Chao1, Observed OTU, and Shannon index) between calves fed selenium-biofortified alfalfa hay compared with control calves showed that Se-supplementation tended to be associated with an enriched nasal microbiota. ANOSIM of unweighted UniFrac distances showed that calves fed high Se-biofortified alfalfa hay clustered separately when compared with control calves in the PCoA plot ($R = 0.216$, $P = 0.04$). The bacterial orders Lactobacillales and Flavobacteriales were increased in healthy control calves compared with Clostridiales and Bacteroidales being increased in calves fed Se-biofortied alfalfa hay. Although there were strong trends, no significant differences were noted for any of the bacterial taxa. Based upon these findings, we suggest that weaned beef calves fed Se-biofortified hay tend to have an enriched nasal microbiota. Feeding Se-biofortified alfalfa hay to weaned beef calves prior to entering the feedlot is a strategy for increasing nasopharyngeal microbial diversity.

Additional publications are in progress as assays are completed. In addition, we will use these results as pilot data to submit future grants for larger funding amounts from the USDA to advance our overall goal of improving the production and health of beef cattle in Oregon by increasing the practice of Se fertilization.



Results of this study will be used as evidence for hay producers in Oregon to adopt the practice of Se-fertilization of forages to provide an enhanced quality of hay, which will then be used to benefit performance and health of beef cattle. This is an innovative and economically viable way of supplementing Se to cattle in our Se-deficient state that we hope will be adopted by hay producers and cattle producers. These results will be shared via peer-reviewed publication, at state meetings, e.g., the Oregon Cattlemen's Association annual meeting, and through extension work and publications.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:
We received funding from the Oregon Beef Council to answer the question: Does feeding selenium fertilized alfalfa hay for eight weeks decrease gastrointestinal parasite load in weaned beef calves?

FUTURE FUNDING POSSIBILITIES:
We have two pending grant proposals: 1) USDA FY16 Animal Health and Disease Program: Feeding cows and calves Se-biofortified hay: Effects on health and disease, and 2) Agricultural Research Foundation: How is selenium (Se) utilized by forage plants after Se is applied to soils as a fertilizer amendment?