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INTERIM REPORT
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TITLE: How does prior selenium supplementation of calves affect immune biomarkers in the feedlot?

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SUMMARY:

Optimal immune function is critical for calves experiencing the stress of weaning, relocation to feedlots, and possibly commingling with animals of different origins. The National Agriculture Statistics Service shows that 630,000 calves were born in Oregon in 2011. 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.

Selenium (Se) is an essential micronutrient in cattle, and Se deficiency can affect morbidity and mortality. Calves may have greater Se requirements during periods of stress. Providing adequate Se is important to prevent Se-responsive diseases in growing cattle such as nutritional myodegeneration (white muscle disease) and Se-responsive unthriftiness. Oregon is known to have soils deficient in Se and, thus, livestock consuming forages raised on these soils will likely suffer Se deficiency. 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. We believe that increasing the bioavailable concentrations of Se in forages through the use of Se fertilizer is the most economical and practical method to provide Se supplementation to cattle. This practice has been demonstrated to be both effective and safe in Finland, New Zealand, and Australia. We believe it can be readily adapted to Oregon cattle production systems. In the United States, the use of feedstuffs that are naturally high in Se content is not regulated; Se fertilization, however, is not allowed in any state except for Oregon, where the Department of Agriculture does not control the use of Se as a plant fertilizer. Therefore, in Oregon it is possible to produce forage crops with increased Se concentration by applying Se as a fertilizer, which is then incorporated into the forages ultimately destined to be fed to cattle. Our goal is

to provide solid data on the health and performance benefits, and on economic returns, so that this practice becomes routine in Oregon.

We completed a pilot study using calves from the Oregon State University Beef Ranch, Corvallis, OR. Sixty weaned beef calves were randomly assigned to 4 groups and fed alfalfa hay for 7 weeks. The alfalfa was harvested from fields fertilized with sodium selenate at a rate of 0, 22.5, 45.0, or 89.9 grams of Se per hectare. Blood samples were collected weekly from these calves and analyzed for whole blood (WB) Se concentrations. Fertilizing with increasing application rates of Se resulted in corresponding increases in alfalfa hay Se content for that cutting of alfalfa (0.07, 0.95, 1.55, 3.26 ppm on a dry matter basis). Subsequent feeding of this Se-fertilized alfalfa hay to the weaned calves increased WB-Se concentrations depending on the Se-fertilization rate. Feeding Se-fertilized alfalfa hay also increased body weight, increased calf survival rates in the feedlot, and increased slaughter weights. The results of the completed pilot study suggest that soil-Se fertilization is a practical management tool to increase forage-Se content in areas with low soil-Se concentrations, and that subsequent feeding of Se-enriched forage leads to improved Se-status and performance in Se-replete calves.

Thus, we have shown that fertilizing alfalfa hay with sodium selenate increases Se content of alfalfa hay in a dose-dependent manner. Feeding that Se-biofortified alfalfa hay to calves increases WB-Se concentration in a dose-dependent manner. The storage form of Se in plants is selenomethionine, which when consumed by the calf is incorporated into many tissue proteins in place of the amino acid methionine. The tissue concentration of selenomethionine ultimately reflects dietary intake. Selenomethionine acts as a storage form of Se in body proteins, from which it is slowly released over time, for example later in the feedlot period when Se intake may be inadequate.

This proposal is dependent on completing a backgrounding feeding period whereby supranutritional concentrations of organic Se in alfalfa hay are fed to enhance Se bioavailability. Based on the pilot study, we expect that supranutritional concentrations of organic Se in the diet will increase WB-Se concentrations, increase body weight gain, and decrease morbidity and mortality in Se-supplemented calves compared to those measured in calves not receiving Se-biofortified hay in an 8-wk backgrounding period. 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.

Once the backgrounding period is complete, our goal is to identify biomarkers in the immune system that correlate with increased susceptibility to disease and can be measured to demonstrate protective effects of Se. We believe Se protection is by a dosage phenomenon and we wish to determine the specific level(s) of Se-biofortified hay needed to raise tissue Se levels to adequately boost immune responses and protect calves from disease in the feedlot. Historically, calves are most susceptible to disease outbreaks between one and two weeks after relocation to the feedlot. Therefore, we will measure Se levels and immune function one and two weeks after transfer to the feedlot and correlate Se measures and immune biomarkers that are affected in acute phase illness. Dosing information can then be shared with producers who are backgrounding weaned beef calves for the feedlot.

OBJECTIVES:

The objective of this study is to determine whether weaned beef calves that received supranutritional concentrations of organic Se in alfalfa hay for 8-wk in the backgrounding period have altered Se-related immune biomarkers one and two weeks after transport to a feedlot. We hypothesize that calves receiving Se-biofortified alfalfa hay during the backgrounding period will have lower concentrations of ROS reflected as higher levels of reduced and total glutathione in erythrocytes; higher levels of circulating selenoprotein P; and lower levels of IL-13 (immunosuppressive cytokine) compared to those measured in calves not receiving Se-biofortified hay.

PROCEDURES:

1. The backgrounding weaned-beef calf study will be completed first, and then we will study the calves after arrival at the feedlot. We will seek the cooperation of feedlot managers to follow these calves into the feedlot for two blood collections.

2. In the backgrounding study, calves will be weaned and randomly assigned to 9 groups of 5 calves each, balanced by body weight, sex, and breed type. 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). Three pens of calves will be fed alfalfa hay as a major portion of the ration plus a mineral supplement containing 120 mg/kg Se (US FDA regulations) from sodium selenite (control group). Three pens of calves will be fed alfalfa hay harvested from a field fertilized with a medium level of Se (M-Se) and a mineral supplement without added Se. Three pens of calves will be fed alfalfa hay harvested from fields fertilized with a high level of Se (H-Se) and fed mineral supplement without added Se. After a period of acclimation all calves will receive an equivalent amount of grain as a minor part (approximately 10% dry matter basis) of the ration. Calves will be maintained on their respective diets for 8 wk.

3. For the 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 M-Se and H-Se forage. In our pilot 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 (control, M-Se, and H-Se). This sampling regime will be repeated 3 times for each alfalfa hay source.

4. Calves will be bled at the beginning of the treatment period (day 0) and bi-weekly for 8 wk 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-wk feeding period to determine weight gain. Health records will be maintained and evaluated to monitor health status during the feeding trial. They will include notations on disease syndromes (off feed, fever, BRD, diarrhea, abscesses, pink eye, etc.) resulting in 'pulls', treatments administered, and response to treatment.

5. In addition, we plan to investigate the mechanisms of Se-induced immune responses in weaned beef calves in the backgrounding period following dietary supplementation with supranutritional concentrations of organic Se by measuring the immune response to multiple vaccines (UPJOHN J-5 BACTERIN™, to ONE SHOT®, and to several of the viral antigens in Bovi-Shield GOLD® 5). We

hypothesize that humoral immunity will be enhanced after short-term exposure to high-Se-fertilized forage. In addition, we will characterize subsets of leukocyte populations (e.g., T and B lymphocytes, macrophages, granulocytes) by flow cytometry to determine what effect consumption of Se-enriched forage has on white blood cell populations. We will also isolate T cells and measure their response to viral antigens contained in the Bovi-Shield GOLD5® vaccine. We hypothesize that supranutritional concentrations of organic Se in the diet will increase T cell activation.

6. Next we will study the calves after arrival at the feedlot. We will study immune responses in these same calves that have been fed Se-biofortified alfalfa hay in the 8-wk backgrounding program following transport to the feedlot. Stress of relocation to the feedlot may be linked to increased generation of harmful reactive oxygen species (ROS), the removal of which requires glutathione and Se. Oxidative stress may contribute to immunosuppression and increased disease susceptibility. Although ROS and free radicals are a natural result of the body's normal metabolic activity, excessive stress as a result of transportation, environmental changes, comingling with other animals, and disease can lead to over-production of free radicals or accumulation of free radicals because of a lack of free radical scavenging antioxidants. Oxidative stress is a primary mechanism by which ROS influence biological processes, and contribute to the pathogenesis of disease by modifying the expression of pro-inflammatory genes. The beneficial effects of Se supplementation may result from selenoproteins reducing harmful ROS to less-reactive molecules.

7. Serum Se concentrations are known to decline during critical illness and are indicative of a poor prognosis. Serum Se is in highest concentration in selenoprotein P, a protein produced in the liver that is involved in Se homeostasis and transport of Se to tissues, and in controlling the expression of antioxidant selenoproteins. Down regulation of the synthesis of selenoprotein P because of inflammation, or its removal from circulation, are the reasons why serum Se concentrations decrease in critical illness. Therefore, quantification of the Se transport protein selenoprotein P may be a more useful biomarker in critical illness than measuring total serum Se. In addition to selenoprotein P, we will also measure the immunosuppressive cytokine (IL-13). IL-13 is known to suppress macrophage function and we can therefore determine if IL-13 levels correlate with serum Se and/or selenoprotein P concentrations.

8. We will bleed these same calves 1 week and 2 weeks after transport to the feedlot to collect blood for the assays: oxidized/reduced/total glutathione in erythrocytes and plasma (OxiSelect™ Total Glutathione Assay Kit; Cell BioLabs, Inc.; San Diego, CA); selenoprotein P (immunoluminometric sandwich assay that uses two polyclonal antibovine selenoprotein P antibodies); and IL-13 (Bovine IL-13 ELISA Kit, Bethyl Laboratories, Inc.; Montgomery, TX). Biomarkers will be correlated to WB-Se status and morbidity and mortality in the feedlot.

9. Statistical analyses: The number of animals assigned to each group (n=15) was chosen based on a previous study, where we calculated significance in blood Se concentrations at the $P<0.01$ level between groups treated with different forms of Se. Data will be reported as least square means ± SEM, and evaluated using an ANOVA method for repeated measures and Statistical Analysis Software [SAS]. Significance will be accepted at $P\leq 0.05$.

SIGNIFICANT ACCOMPLISHMENTS:

Sodium selenate mixed with water was sprayed onto the soil surface of an alfalfa field after the second cutting of hay in summer 2014. Hay was made and delivered to the metabolism barn in the fall of 2014. Cows are calving and raising the calves that will be weaned in the Fall 2015 and used in this project.

Two papers from the pilot study have been published:

Hall JA, Bobe G, Hunter JK, Vorachek WR, Stewart WC, Vanegas JA, Estill CT, Mosher WD, Pirelli GJ. Effect of feeding selenium-fertilized alfalfa hay on performance of weaned beef calves. **PLoS ONE** 2013;8(3):e58188. doi: 10.1371/journal.pone.0058188. [Epub 2013 Mar 11].

Hall JA, Bobe G, Vorachek WR, Hugejiletu, Gorman ME, Mosher WD, Pirelli GJ. Effects of feeding selenium-enriched alfalfa hay on immunity and health of weaned beef calves. **Biol Trace Elem Res** 2013; 156(1-3):96-110. doi: 10.1007/s12011-013-9843-0. [Epub 2013 Oct 20].

BENEFITS & IMPACT:

We expect to provide convincing evidence that Se supplementation with high-Se-fertilized forage is beneficial for enhancing immune biomarkers in beef calves one and two weeks after transport to a feedlot. This will have a significant economic benefit to Oregon ranchers who adopt this strategy.

ADDITIONAL FUNDING RECEIVED:

This study was dependent on receiving funding from a concurrent proposal submitted to the Oregon Beef Council. However that study was not funded, which delayed the completion of this proposal. Because this proposal looks at the time frame just after arrival in the feedlot, we had to find additional funding to complete the backgrounding part of the study. We have leftover hay from our pregnant beef cow/calving study which we plan to feed these calves in the 8-wk backgrounding period.

We anticipate receiving additional funding from an alfalfa hay grower in central Oregon in the form of alfalfa hay that we can sell to fund our research. We are just beginning these discussions and have met with Dr. Kelvin Koong in February 2015 to discuss how to process product and/or funds.

FUTURE FUNDING:

I submitted the following grant as Program Director in Spring/Summer 2014 based, in part, on this project, entitled “Selenium: Supranutritional agronomic biofortification to enhance immune function and disease prevention” to the National Institute of Food and Agriculture; Agriculture and Food Research Initiative (AFRI): Food Security Challenge Area: Minimizing Losses from Pests and Diseases of Livestock: A5152, $4,770,363.00. 2015-2019. Unfortunately only one proposal was funded (not ours). We will resubmit as funding opportunities arise.