AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Does Feeding Selenium-Fertilized Alfalfa Hay to Pregnant Beef Cows in the Third Trimester Increase Passive Absorption of Immunoglobulin G in Newborn Calves & Improve Performance?

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SUMMARY:

Our goal is to prevent diseases in cattle by optimizing immune function with supranutritional selenium (Se) 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 an essential micronutrient for cattle affecting both performance and immune functions. Severe Se deficiency results in nutritional myopathy or "white-muscle disease", whereas subclinical Se deficiency causes muscular weakness of the newborn, immunosuppression, unthriftiness, reduced weight gain, and scours in calves. Adequate Se must be provided to prevent Se-responsive diseases related to a deficiency of Se in the diet. Providing Se to the mother during gestation is an effective method to meet Se requirements in the newborn because Se efficiently crosses the placenta into fetal tissues and enters colostrum and milk.

Current FDA regulations limit the amount of dietary Se supplementation in ruminant animals to 0.3 mg/kg (as fed) of inorganic sodium-selenite or sodium-selenate, or organic Se-yeast, which is equivalent to 3 mg per beef cow per day. Another way to provide Se is by increasing its content in forages. Selenium fertilization has been shown to enhance Se levels in feedstuffs, including alfalfa hay, and in animals and humans consuming such feedstuffs. Several countries with low soil-Se concentrations including Finland, Denmark, New Zealand, and the United Kingdom use Se fertilization to increase Se concentrations in the food chain. In the US, Se fertilization is only allowed in Oregon. The Department of Agriculture does not control the use of Se as a plant fertilizer; therefore, it is possible to produce feedstuffs with increased Se concentrations (supranutritional levels) by applying Se as a fertilizer.

There are two means by which an animal gains immunity against an infectious disease. The first, (passive immunity) includes antibodies transferred from the mother to her offspring in colostrum. These passively transferred antibodies provide immediate protection, but are eventually broken down such that as concentrations decline, the animal becomes susceptible to infection. The second, (acquired immunity) involves administering an antigen to an animal, who responds by developing a primary immune response. The disadvantage of acquired immunity is that protection is not available immediately; however, once established acquired immunity lasts a long time. Antibodies first become detectable about one week after vaccination (or exposure to a pathogen) and their concentration in serum increases for 10 to 14 days and then declines. The amount of antibody formed and the amount of protection conferred during the primary response is relatively small. Upon second exposure to the same antigen the response is very different, occurring more quickly, with antibodies reaching higher levels that last much longer. By adulthood, most cattle have been vaccinated or exposed to pathogens, and thus, acquired immunity provides ongoing protection. In contrast, newborn animals are susceptible to diseases that present little threat to adults. Cattle, unlike humans, do not receive passive immunity in utero as antibodies do not across the placenta. Thus, antibodies provided in the colostrum are extremely important for interim protection of the neonate.

In ruminants, immunoglobulin G (IgG) is the predominant immunoglobulin in colostrum. Naturally-suckled calves ingest an average of 2 liters of colostrum. In the digestive tract of newborn calves, colostral proteins are not degraded but instead reach the small intestine intact where they are actively taken up by epithelial cells. They eventually reach the systemic circulation, which allows the newborn to obtain a massive transfusion of maternal immunoglobulins. The period during which the intestine is permeable to proteins varies, but is highest immediately after birth, declines after 6 hours, and drops to relatively low levels by 24 hours. Newborn animals that do not suckle adequately possess low levels of IgG in their serum. Because of the nature of the absorptive process, peak serum IgG concentrations are normally reached between 12 and 24 hours after birth. After absorption ceases, concentrations of these passively acquired antibodies immediately begin to decline through normal body degradation processes. Failure of passive transfer predisposes a young animal to infection.

OBJECTIVES:

1) To determine if feeding pregnant beef cows Se-fortified alfalfa hay in the last 8 wk of pregnancy enhances passive transfer of IgG in beef calves.
2) To determine if there are health benefits (e.g., less disease morbidity and better weight gains) associated with this enhanced passive transfer of IgG in beef calves, from birth to weaning (approximately 6 months).

PROCEDURES:

1. The study design consisted of three groups of beef cows that were housed in the Hogg Animal Metabolism Barn on campus during the last 8 wk of pregnancy. Because pen is the experimental unit in animal nutrition studies, we put 5 cows per pen and had 3 pen replicates for each of three Se-treatment groups (total of 45 cows). **Group 1 (control):** Three pens of cows (n=15) were fed non-Se fortified alfalfa hay as a major portion of the ration plus a mineral supplement containing 120 mg/kg Se (US FDA regulations) from sodium selenite. **Group 2 (M-Se):** Three pens of cows (n=15) were fed alfalfa hay harvested from a field fertilized with a moderate level of Se (M-Se) and a mineral supplement without added Se. **Group 3 (H-Se):** Three pens of cows (n=15) were fed alfalfa hay harvested from fields fertilized

with a high level of Se (H-Se) and fed mineral supplement without added Se. Cows were maintained on their respective diets for 8 wk prior to calving.

2. For the Se-enriched alfalfa forage, sodium selenate was mixed with water and sprayed onto the soil surface of an alfalfa field after the second cutting of hay. Two application rates of selenate were 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 was harvested from the respective field plots, and then sampled for Se content. A Penn State forage sampler was used to take 25 cores from random bales in each alfalfa hay source (control, M-Se, and H-Se). This sampling regime was repeated 3 times for each alfalfa hay source.

3. As cows calved, an aliquot of their first colostrum was saved and frozen for later assays. Calves were allowed to nurse at will and were weighed prior to and immediately after each nursing to estimate the amount of colostrum consumed. Afterwards, cows and calves were returned to the Soap Creek beef ranch.

4. Whole blood samples were collected from all cows prior to feeding Se-fortified alfalfa hay and at parturition to measure blood-Se concentrations. Blood was collected in calves at birth and at 48 hours to measure blood-Se concentrations. Two ml of blood was collected from the jugular vein into EDTA tubes and shipped on ice to Utah Veterinary Diagnostic Lab where Se concentrations were determined using an ICP-MS method. IgG concentrations were determined in serum at birth (none) and at 48 hours. To assess IgG concentrations, serum was collected and titers were assessed in Dr. Hall's lab using an indirect ELISA procedure. The Se and IgG concentrations in colostrum from each cow were also measured.

5. Body weights of all calves were recorded at birth. Production records (weight gains) and adverse health events (scours, infectious disease incidents) were recorded until calves were weaned at approximately 6 months.

6. Objective one was accomplished by determining IgG concentrations in those calves that received colostrum from cows fed Se-fortified alfalfa hay. We hypothesized that passive transfer is enhanced in calves born to cows fed Se-fortified alfalfa hay compared with calves from control cows receiving sodium-selenite in salt. Objective two was accomplished by determining disease morbidity in calves from cows fed Se-fortified alfalfa hay. We hypothesized that calves from cows fed Se-fortified alfalfa hay had fewer disease events and better weight gains compared with calves from control cows receiving sodium-selenite in salt.

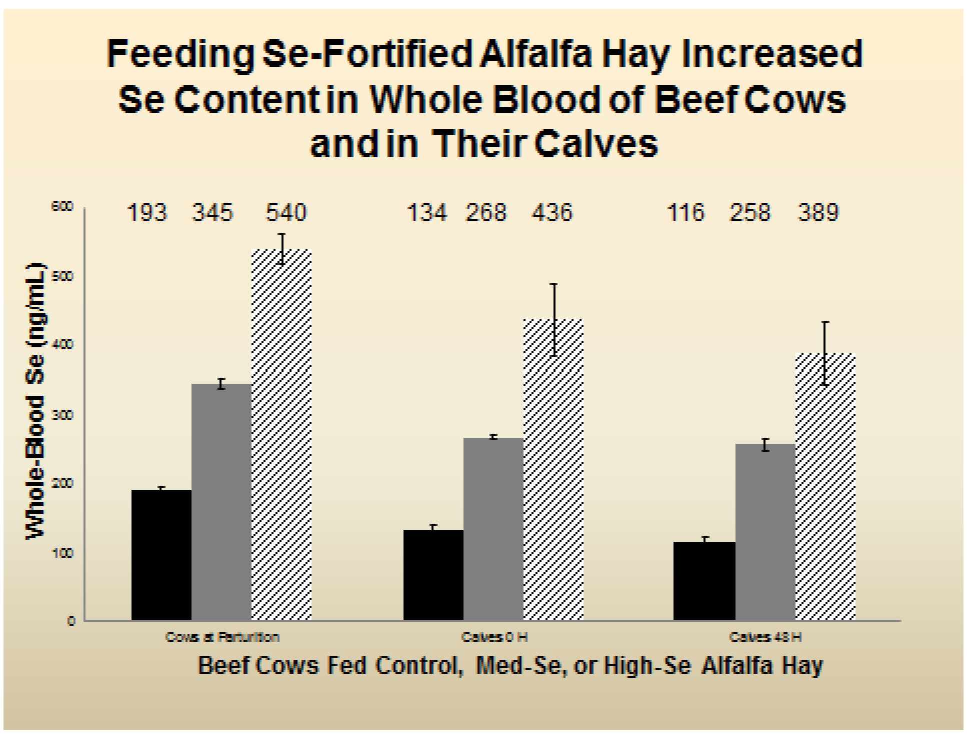
7. Statistical analyses: Whole blood Se, and serum and colostrum IgG concentrations in the calves were evaluated using an ANOVA method for repeated measures and Statistical Analysis Software [SAS]. Significance was accepted at $P \leq 0.05$.

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 were moved into the metabolism barn on the OSU campus in early December 2014 and fed control, medium-Se, or high-Se alfalfa hay until they calved (January 29 to March 7, 2015). Whole blood samples from cows, WB samples from calves, and colostrum samples from cows were collected, processed, and frozen for further analysis. All Se analyses in WB of cows and calves, and in colostrum of cows are completed. Determinations of IgG concentrations in serum of cows and calves, and in colostrum of cows are in progress.

Fertilizing fields with increasing amounts of Na selenate increased the Se-concentration of third cutting alfalfa hay from 0.36 mg Se/kg DM (non-fertilized control) to 2.42 and 5.17 mg Se/kg DM for Na selenate application rates of 45.0 and 89.9 g Se/ha, respectively. The relationship between amount of Se applied by fertilization (g Se/ha) and observed forage Se concentration (mg Se/kg DM) was $y = 0.054x + 0.244$, $R^2 = 0.9931$. Calculated Se intake from dietary sources was 9.0, 32.9, and 70.3 mg Se/head/d for cows consuming hay with Se concentrations of 0.36 to 2.42 and 5.17 mg Se/kg DM, respectively.

Prior to the feeding trial, cows had WB-Se concentrations (mean, 149 ng/mL; range, 106-243 ng/mL), which were within the reference interval of adult cows (120-300 ng/mL). WB-Se concentrations increased after 4 wk of feeding Se-fertilized alfalfa hay depending upon the Se-application rate (0, 45.0, or 89.9 g Se/ha) to 164 ± 6, 253 ± 8, and 339 ± 5 ng/mL ($P_{Linear} < 0.001$), respectively. WB-Se concentrations increased again after 8 wk of feeding Se-fertilized alfalfa hay. The figure below shows Se content in WB of beef cows at parturition (after 8 wk of feeding Se-fertilized alfalfa hay; black bars = cows consuming 9.0 mg Se/head/d; grey bars = cows consuming 32.9 mg Se/head/d; striped bars = cows consuming 70.3 mg Se/head/d) and in WB of calves at birth and in calves at 48 hours after birth. Colostral Se concentration (after 8 wk of feeding Se-fertilized alfalfa hay) were 121 ± 6, 504 ± 9, and 1,339 ± 19 ng/mL ($P_{Linear} < 0.001$), respectively.



BENEFITS & IMPACT:

This proposal benefits Oregon beef producers by validating a Se-supplementation technique for newborn calves. As an immunonutrient, Se may enhance protection of cattle, and as a consequence, improve the Nation's agriculture and food supply. If the incidence or severity of infectious disease can be reduced via Se supplementation, this leads to improved quality of life in rural America in that less labor-intensive management practices would be required, less expense would be involved in treatment of disease, and consequently animal production would improve. The information obtained from this project will be used in educational programs and publicized through producer and public news media.

Preliminary results were presented as an invited talk at the 4th International conference on Selenium in the Environment and Human Health, October 18-21, 2015, Sao Paulo, Brazil. Proceedings were entitled: Strategies for Se supplementation in cattle: Se-yeast or agronomic biofortification.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

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

FUTURE FUNDING POSSIBILITIES:

A grant was submitted in June 2015 based, in part, on this project, entitled "Agronomic selenium biofortification to promote animal health, food quality, and food security" to the National Institute of Food and Agriculture; Agriculture and Food Research Initiative (AFRI): Food Security Challenge Area: Agricultural Production Systems: A5160, $3,998,119.00. 2016-2019. Unfortunately only one proposal was funded (not ours). We plan to resubmit as funding opportunities arise.