Effects of Selenium Supplementation During Different Trimesters of Pregnancy on Total and *Vibrio coralliilyticus* Specific IgM Antibody Concentrations in Beef Cows at Parturition

By

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ABSTRACT

Background: The immune response to microbial infections is compromised in beef cattle at parturition. Innate and adaptive immunoglobulin M (IgM) serve as initial antibodies during microbial infection before the generation of adaptive immunoglobulin G (IgG). Selenium (Se) is an essential micronutrient required by all mammals, which can improve immune responses in beef cattle.

Objective: To determine the best time during pregnancy to supplement beef cows with organic Se to optimize immune function at parturition. We hypothesized that feeding Se yeast to pregnant beef cows in each of the three trimesters of pregnancy would alter total and *Vibrio coralliliiycticus* specific IgM concentrations in cows at parturition.

Animals: A total of 79 black Angus and Angus cross beef cows were used in the study. All of the cattle were housed and obtained routine care including deworming and vaccinations at Oregon State University Soap Creek Ranch, Corvallis OR.

Procedures Twenty-three cows were in the control group; they did not receive any Se-yeast boluses. Twenty-one cows were in Group 1. These cows received three 52.5g Se-Yeast boluses once weekly in the first 3 months of their pregnancy. Fifteen cows were in Group 2. These cows received three 52.5g Se-Yeast boluses once weekly in the second trimester of pregnancy from months three to six. Twenty cows were in Group 3. These cows received three 52.5g Se-Yeast boluses once weekly in the third trimester of pregnancy, from months six through nine. Thus, all cows except controls received 105 mg Se/week from the three boluses administered during their specific treatment trimester, equating to five times the upper limit for US FDA Se administration regulations. Blood was collected at parturition from all cows. A commercial ELISA kit was used to measure total IgM antibody concentrations in serum of beef cows at parturition (Bethyl...
Bovine IgM Kit). To test for *Vibrio coralliilyticus* specific IgM antibody concentrations and whether differences in concentration occur with timing of Se treatments during gestation, an ELISA protocol was also developed for detecting *Vibrio coralliilyticus* specific IgM antibodies.

**Results:** Total and *Vibrio coralliilyticus* specific IgM concentrations were highly correlated (r = 0.78; P < 0.0001). Compared with values of Control cows, cows treated with Se in the first, second, or third trimester of pregnancy, respectively, had similar serum total IgM concentrations (P = 0.43; P = 0.86, and P = 0.19, respectively) and similar *Vibrio coralliilyticus* specific IgM concentrations (P = 0.47; P = 0.55, and P = 0.67, respectively).

**Conclusions and Clinical Relevance:** These findings show that weekly organic Se-yeast supplementation using a 105 mg Se/wk dosage during different trimesters of pregnancy does not affect IgM concentrations in beef cows after parturition. We do not provide evidence for Se-yeast bolus Se supplementation of beef cows during pregnancy impacting IgM production at calving.
INTRODUCTION

Selenium (Se) is an essential micronutrient required by all mammals. The importance of Se was highlighted when it was discovered that a deficiency of Se has an effect on oxidative processes and cell membrane protection (Rotruck, 1973). Selenium's function in a mammalian body is critical to produce antioxidant enzymes whereby the enzymes break down peroxides; these peroxides can cause DNA and tissue damage (Giadinid et al., 2012) and result in illnesses. The lack of Se in ruminant diets, e.g., in beef cattle, can be detrimental to their health and their owner’s livelihood. A deficiency of Se can cause diseases such as white muscle disease (nutritional myodegeneration) in which fibrous connective tissue and calcium salts are dispersed among the muscle fibers in heart and skeletal muscle (Perez et al., 2010). Additionally, a deficiency of Se can lead to impaired immune responses and diseases such as mastitis in the perinatal period, and alter the milk quality in cows (Mehdi, 2016). Blood concentrations of IgG and T cell functions can also be affected by Se deficiency because of increased oxidative stress (Zhi et al., 2012). For example, Se supplementation increases the number of T cells, thus enhancing this immune response (Wood et al., 2000).

The immune system does a phenomenal job at protecting us from disease, however nutrient deficiencies can affect how our immune system functions. Immunoglobins consisting of IgA, IgG, IgM, IgD and IgE play a key role in how the immune system works. IgA compromises about 15% of the total immunoglobulins in blood, saliva, respiratory and gastric secretions. IgG makes up about 70-80% of the immunoglobulins in the blood, and these antibodies provide long term protection against microorganisms (Merk, 2010). IgM antibodies are essential because they are involved in the body’s first response to a new infection. The IgM structure is the largest of
the antibodies and are differentiated by innate (or natural) IgM, which are synthesized by innate-like B-1 cells without pathogen exposure, and adaptive (or immune) IgM, which are synthesized by innate-like B-1 and adaptive B-2 cells after pathogen exposure (Thompson et al., 2020).

To evaluate whether organic Se supplementation of pregnant beef cows plays a role in humoral immunity, total and *Vibrio coralliilyticus* specific IgM antibody concentrations were determined. These assays test for natural antibodies (IgM) that recognize conserved sequences in nature. Natural antibodies are present in all animal's and provide them with innate immune protection (Dugovich et al., 2017). These IgM antibodies are also known to have functional roles in the immune responses, such as suppression of allergic responses and regulation of B cell responses (Hernandez et al., 2017). These functional roles help to maintain physiological homeostasis and protect against bacterial infections (Chen et al., 2009).

The overall objective of this study was to determine the best time during pregnancy to supplement beef cows with organic Se to optimize cattle health and calf production. Although ELISA kits are available to measure total IgM antibody concentrations in beef cows (Bethyl Bovine IgM Kit), in order to test for *Vibrio coralliilyticus* specific IgM antibody concentrations and whether differences in concentration occur with timing of Se treatments, a protocol had to be developed for detecting *Vibrio coralliilyticus* specific IgM antibodies in beef cows (*Bos Taurus*). A similar protocol had been published for a related ruminant species, i.e., in Bighorn Sheep: “Detection of bacterial-reactive natural IgM antibodies in desert bighorn sheep populations” (Dugovich et al., 2017.) We hypothesized that a weekly Se-yeast bolus treatment of
pregnant beef cows in each of the three trimesters of pregnancy would alter natural and *Vibrio coralliilyticus* specific IgM concentrations in cows at parturition.
MATERIALS AND METHODS

The experimental procedure was reviewed and approved by the Oregon State University Animal Care and Use Committee (Animal Care and Use Proposal number 0056). Angus and Angus cross cows were assigned to one of four treatment groups (control, and groups one, two and three, corresponding to their Se treatment trimester) after conception in a randomized complete block design, 15 to 25 cows per group. All 79 cows had calved at least once previously. Cow ages ranged between three and eleven years. All cows scored a four out of five on the body condition scoring scale. The cows were bred to one sire using sexed semen and artificial insemination. Several bulls were then used to inseminate cows that did not become pregnant by artificial insemination.

Animals

A total of 79 black Angus and Angus cross beef cows were used in the project. Twenty-three cows were in the control group; they did not receive any Se-yeast boluses. Twenty-one cows were in Group 1. These cows received three 52.5g Se-Yeast boluses once weekly in the first 3 months of their pregnancy. Fifteen cows were in Group 2. These cows received three 52.5g Se-Yeast boluses once weekly in the second trimester of pregnancy from months three to six. Twenty cows were in Group 3. These cows received three 52.5g Se-Yeast boluses once weekly in the third trimester of pregnancy, from months six through nine. All of the cattle were housed and obtained routine care including deworming and vaccinations at Oregon State University Soap Creek Ranch, Corvallis OR.

During their assigned pregnancy trimester, cows received Se-supplementation via three boluses of Se-yeast, administered orally with a balling gun once per week for 13 weeks. All cows
except controls received 105 mg Se/week from the three boluses administered during their specific treatment trimester, equating to five times the upper limit for US FDA Se administration regulations. All cows were also given free-choice access to a mineral supplement containing 120 mg/kg Se (US FDA regulations) from sodium selenite.

**Serum Sample Collections**

Whole blood samples were collected from the jugular vein of cows at baseline, at the beginning of each trimester of pregnancy, and at parturition (within the first 48 hours postpartum) as part of a larger experimental design. Blood was collected using red top (no anticoagulant) evacuated blood collection tubes. These samples were centrifuged at 2500 RPM for 15 min, and serum was then transferred into 2.0 mL screw cap self-standing micro tubes (ISC BioExpress, Kaysville, UT). The samples were then frozen at -20 °C until further analysis was performed.

**Vibrio coralliilyticus Culture and Cell Envelope Isolation**

*Vibrio coralliilyticus* strain was provided by Brian Dolan from Oregon State University.

**ELISA Protocol Development**

In order to measure *Vibrio coralliilyticus* specific IgM antibody concentrations, an ELISA protocol had to be developed for the bovine species. Using a previous study “Detection of bacterial-reactive natural IgM antibodies in desert bighorn sheep populations” (Dugovich et al., 2017), we created a new ELISA protocol for beef cows. Despite this article using a ruminant specimen, there were still unknown parameters that had to be evaluated such as bovine sera
dilutions, conjugated-detection antibody dilutions, and *Vibrio coralliilyticus* membrane protein dilutions.

Previously collected cow sera was used for protocol development. Serum samples were analyzed in duplicate for each cow. A 96 well-plate was coated with 100 µl of 1:2000 dilution of *Vibrio coralliilyticus* membrane protein and was left to rest overnight in a refrigerator. After overnight incubation, the plate was then washed 6 times with a PBS+Tween 20 buffer solution by hand and then blocked with 300 µl of 1% bovine serum albumin (BSA; Bethyl Laboratories Inc, Montgomery, TX) for 1 hour at room temperature. Plates were then washed 6 times with PBS+Tween 20 buffer solution to remove the blocking solution.

Next, 20 µl of cow serum was serially diluted in duplicate to 1:10, 1:100, 1:10,000, 1:100,000, 1:200,000, 1: 400,000 using PBS for serial dilutions. Then 100 µl of diluted serum was added to each well of the ELISA plate and incubated at room temperature for one hour to bind to the plate-bound *Vibrio coralliilyticus* membrane protein. After the incubation period, the plate was washed six times with buffer solution to remove any unbound serum. Next, 100 µl of a 1:20,000 and 1:200,000 dilution of sheep anti-bovine IgM biotinylated (Bethyl Laboratories Inc, Montgomery, TX) solution (diluted in 1% BSA) was added to each well and the plate was left to incubate at room temp for 1 hour for the sheep anti-bovine IgM to bind to the sera IgM that had bound to bacterial proteins of *Vibrio coralliilyticus*. The plate was then washed 6 times with buffer solution and 100 µl of Streptavidin HRP (Life Technologies, Burlington, ONT) in a 1:3000 dilution in 1% BSA was added to each well and incubated for 45 minutes at room temperature. Afterwards the plate was washed 5 more times, and then 100 µl of TMB (3,3′, 5,5″-
tetramethylbenzidine; BioLegend, San Diego, CA) was added to each well. No reaction occurred in any of the wells including the negative control wells.

A new ELISA protocol was subsequently developed, following the same steps as referenced above, but with different concentrations and times. For example, a 1:20 Vibrio coralliilyticus dilution was created and used to coat the 96 well plate. Three cow serum samples were tested in duplicates consisting of the following serial dilutions: 1:10, 1:100, 1:1000, 1:10,000, 1:100,000. Then a 1:200 dilution of the sheep anti-bovine IgM biotinylated was prepared by diluting in 1% BSA. The ELISA plate color was read at an absorbance measurement of 450 nm using a Synergy Microplate Reader (BioTek, Winooski, VT). It was determined that the 1:10 and 1:100 sera dilutions were too concentrated, and that the best dilutions of sera were between 1:1000 and 1:10,000 so it was decided to increase the number of dilutions within that range.

In order to decrease background noise, a 1:50 Vibrio coralliilyticus dilution was created to coat the 96 well plate, and 1:500, 1:1000, and 1:1500 cow sera dilutions were made from the 3 cows’ sera, in duplicate. A 1:200 dilution of sheep anti-bovine IgM biotinylated diluted in 1% BSA was also used. The plates were then read, and it was determined that the 1:500 and 1:1000 serum dilutions were the best sera dilutions.

In order to further reduce background noise, we tweaked the dilutions of conjugated-detection antibody of sheep anti-bovine IgM biotinylated. Dilutions of 1:200 and 1:500 were compared while keeping all other conditions and steps the same. After reading the plate it was
seen that the 1:500 dilution of sheep anti-bovine IgM biotinylated was best because there was less background noise.

The concentration of *Vibrio coralliilyticus* specific IgM antibodies present in sera was calculated from a standard dilution series. A standard curve was constructed by plotting absorbance against the concentrations of the standard solution.

**Vibrio coralliilyticus Specific IgM Concentrations in Beef Cow Sera at Parturition**

Concentrations of *Vibrio coralliilyticus* specific IgM were measured in cow sera collected at parturition from 10 cows in the control group and 10 cows in each of the three Se treatment groups using the validated ELISA assay described above. These cows were randomly selected at the beginning of the study for repeated measures. Serum samples were analyzed in duplicate for each cow.

In brief, two 96-well plates were coated with a 1:50 dilution of *Vibrio coralliilyticus* membrane protein in approximately 100µl of PBS and left in a refrigerator overnight. The ELISA plates were then washed 6 times with a PBS+Tween 20 buffer solution by hand and then blocked with 300 µl of 1% bovine serum albumin (BSA; Bethyl Laboratories Inc, Montgomery, TX) for 1 hour at room temperature. Plates were then washed 6 times with PBS+Tween 20 buffer solution to remove the blocking solution. Next, 20 µl of cow serum was serially diluted in duplicate to 1:1000 and 1:2000 using PBS+tween 20. Then 100 µl of diluted serum was added to a well of the ELISA plate. Once all sera were added, the plate was incubated at room
temperature for one hour for *Vibrio coralliilyticus* specific IgM in sera to bind to the *Vibrio coralliilyticus* membrane protein on the plate. After the incubation period, the ELISA plates were washed five times with buffer solution to remove unbound serum. Next, 100 µl of a 1:500 dilution of sheep anti-bovine IgM biotinylated (Bethyl Laboratories Inc, Montgomery, TX) diluted in 1% BSA was added to each well and was left to incubate at room temp for 1 hour to bind to the sera IgM that was bound to bacterial proteins of *Vibrio coralliilyticus*. The plates were then washed 5 times with buffer solution. Next 100 µl of Streptavidin HRP (Life Technologies, Burlington, ONT) in a 1:3000 dilution in 1% BSA was added to each well and incubated for 45 minutes at room temperature. Afterwards the plates were washed 5 more times, and then 100 µl of TMB (3,3′, 5,5′-tetramethylbenzidine; BioLegend, San Diego, CA) was added to each well. A reaction between TMB and HRP occurs causing a measurable blue color change that correlates with analyte level. After visualizing a blue hue color, the reaction was stopped with 100 µl of ELISA stop solution consisting of 0.18 M of H₂SO₄. Lastly, the ELISA plate color was read at an absorbance measurement of 450 nm using a Synergy 2 Microplate Reader (BioTek, Winooski, VT).

**Total IgM Concentrations in Beef Cow Sera at Parturition**

Beef cow IgM concentrations were evaluated using a Bovine IgM ELISA Kit (Bethyl Laboratories, Montgomery, TX) following manufacturer’s instructions. Serial dilutions of standard at 1:1000, 1:500, 1:400, 1:250, 1:200, 1:100, 1:50 were performed to prepare a standard curve for IgM. Serum samples were diluted in duplicate to 1:20,000.
**Data Analysis**

All analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA) and SAS 9.4 (SAS Institute Inc., Cary, NC). For both IgM assays, a standard curve was fitted using a sigmoidal 4-parameter logistic equation in GraphPad Prism to determine concentrations of the serum samples. Figures were created in GraphPad Prism. PROC GLM in SAS was used to determine the effect of Se supplementation trimester on serum IgM values. Each Se supplementation group was compared with the Control group, which did not receive Se boluses. Pearson and Spearman correlation coefficients in PROC CORR in SAS were used to examine the relationship between total IgM and *Vibrio coralliilyticus* specific IgM. All statistical tests were two-sided. Statistical significance was determined at $P < 0.05$. 
RESULTS

*Development of *Vibrio coralliilyticus* Specific IgM Assay*

The following conditions gave the best color development results: *Vibrio coralliilyticus* cell envelope proteins as an antigenic target for IgM at a dilution of 1:50. Best serum dilutions were determined to be 1:1,000 and 1:2,000. Best color development for serum samples with least background color development was obtained with biotinylated sheep anti-bovine IgM at a dilution of 1:500.

A pooled serum sample from all cows was used for the standard curve. An aliquot of serum from each cow was contained in the pooled sample. Then a set of dilutions ranging from 1:1000, 1:1500, 1:2000, 1:3000, and 1:4000 was prepared to create a standard curve, which was run on each ELISA 96 well plate (*Fig. 1a and 1b*). The standard curve was then used to determine the *Vibrio coralliilyticus* specific IgM values from each of the test cows.

![Standard Curve Plate 1](image1)

![Standard Curve Plate 2](image2)

*Fig 1:* Standard curves of cow sera at parturition for IgM binding to *Vibrio coralliilyticus* membrane proteins were prepared from 10 aliquots of sera from control group of cows and 10 aliquots of sera from each of the three Se trimester treatment groups of cows. Samples were tested in duplicate. For curve-fitting, a sigmoidal 4-parameter logistic equation was used. The standard curve for plate 1 had an $R^2 = 0.9971$ and the standard curve for plate 2 had an $R^2 = 0.9893$. 

1a)

1b)
**Vibrio coralliilyticus Specific IgM Concentrations in Beef Cow Sera**

To evaluate whether organic Se supplementation and/or time when Se supplementation was administered during gestation in beef cows affects *Vibrio coralliilyticus* specific IgM antibody concentrations after parturition, the ELISA developed above was performed on serum samples: For the Control, 1st trimester, 2nd trimester, and 3rd trimester group, 11, 9, 10, and 10 cows were randomly selected and samples were analyzed in duplicate (Fig. 2). A pooled serum sample was used for the standard curve. *Vibrio coralliilyticus* specific IgM antibody concentrations after parturition varied between 0.75 to 4.17 relative to the pooled serum sample. Cows treated with Se in the first, second, or third trimester of pregnancy, respectively, had in serum similar *Vibrio coralliilyticus* specific IgM antibody concentrations after parturition compared with Control cows ($P = 0.43$; $P = 0.86$, and $P = 0.19$, respectively).

*Fig 2:* An ELISA for detecting cow serum IgM binding specifically to *Vibrio coralliilyticus* membrane protein was conducted on 40 serum samples: 11 from control and 9 or 10 from each Se trimester treatment group of cows. All of the samples were analyzed in duplicate. Results are shown for each group of cows as a scatterplot with median and interquartile range indicated. Cows treated weekly in the first, second, or third trimester, respectively, with Se-yeast
boluses had similar serum *Vibrio coralliilyticus* specific IgM concentrations compared with Control cows ($P = 0.43$, $P = 0.86$, and $P = 0.19$, respectively).

**Total IgM Concentrations in Beef Cow Sera at Parturition**

To evaluate whether organic Se supplementation and/or time when Se supplementation was administered during gestation in beef cows affects total serum IgM concentrations, the Bethyl IgM kit was used to measure total IgM in cow serum samples. The same cows were selected for total and *Vibrio coralliilyticus* specific IgM concentrations. A commercial standard from the Bethyl IgM kit was used for the standard curve. Total IgM antibody concentrations after parturition varied between 1.84 and 8.99 mg/mL. Cows in the first trimester had total serum IgM concentrations of $3.18 \pm 0.51$ mg/mL relative to control cows having $4.38 \pm 0.48$ mg/mL ($P$-value: 0.10). Cows in the second trimester had total serum IgM concentrations of $4.32 \pm 0.51$ mg/mL ($P$-value: 0.94 relative to the control cows), and cows in the third trimester had total serum IgM concentrations of $3.58 \pm 0.48$ mg/mL ($P$-value: 0.25 relative to control cows).

**Fig 3:** An ELISA for detecting total cow serum IgM concentrations using the Bethyl IgM ELISA kit was conducted on 40 serum samples: 11 from control and 9 or 10 from each Se trimester treatment group of cows. All of the samples were analyzed in duplicate. Results are shown for each group of cows as a scatterplot with median at interquartile range indicated. Cows in the first trimester had similar serum total IgM concentrations relative to the
control cows with $P$-value: 0.10, cows in the second trimester $P$-value: 0.94, and cows in the third trimester $P$-value: 0.25.

Correlations between total and *Vibrio coralliilyticus* specific IgM concentrations were $r_{\text{Pearson}} = +0.78$ ($P < 0.0001$) and $r_{\text{Spearman}} = +0.76$ ($P < 0.0001$). Whole-blood Se concentrations at parturition were not significantly associated with total IgM concentrations ($r_{\text{Pearson}} = +0.06; P = 0.74$ and $r_{\text{Spearman}} = +0.15; P = 0.36$) and *Vibrio coralliilyticus* specific IgM concentrations ($r_{\text{Pearson}} = +0.04; P = 0.81$ and $r_{\text{Spearman}} = +0.07; P = 0.68$) in serum.
DISCUSSION

Our results show that IgM antibody concentrations were similar amongst groups of cows. We hypothesized that feeding Se yeast to pregnant beef cows in each of the three trimesters of pregnancy would alter IgM concentrations in cow serum at parturition. However, all Se-treatment groups had similar median/range IgM concentrations compared with the control group of cows. In addition, all Se-treatment groups of cows had similar serum *Vibrio coralliilyticus* specific IgM concentrations relative to the pooled sample as did control cows, meaning that the amount of natural IgM antibodies binding to the bacterial membrane protein of *Vibrio coralliilyticus* was similar in each Se-treatment group. This shows that total and *Vibrio coralliilyticus* specific IgM concentrations are not altered by Se yeast supplementation of pregnant beef cows. The concentration of total IgM in our cows ranged from 1.83 to 8.99 mg/mL with a mean and standard deviation of 3.87 ± 1.55 mg/mL. These results are comparable with those reported by others. Serum IgM concentrations of periparturient cows with less than three pregnancies was 7.3 ± 4.6 mg/mL, whereas cows with one or two pregnancies had IgM concentrations of 4.7 ± 3.2 mg/mL (Herr et al., 2011).

Natural IgM antibodies are involved in innate immune responses and are able to recognize conserved epitopes present in pathogens (Dugovich et al., 2017). These IgM antibodies are different from adaptive antibodies, which are specific to particular pathogens that the host has been previously exposed to. However, IgM antibodies are not non-specific, as they recognize conserved antigen epitopes present on multiple microbial agents (reviewed in Dugovich et al., 2017). As such, binding of IgM antibodies to foreign antigen provides the body’s first line of defense to a new infection (Gunti et al., 2015). Furthermore, this allows
antigens bound to IgM antibody to interact with other components of the immune system (Ehrenstein et al., 2010). The overall goal is to provide early protection against organisms that are bacterial, viral, parasitic, or fungal in nature. Because of its low affinity and high valency, IgM is capable of enhancing pathogen agglutination and neutralization (Quartier et al., 2005).

Natural antibodies are mostly of the IgM subclass of antibodies. We chose to look at natural antibodies to cell envelope proteins of *Vibrio coralliilyticus*, a bacterial pathogen that infects corals and oyster. Because cattle have not been exposed to this bacterium, no acquired antibody is present to cross-react with the bacterial targets. The goal was to measure the cows’ ability to withstand novel infections based on Se-status. Because natural antibodies to *Vibrio coralliilyticus* are usually in the IgM subclass of antibodies, it is possible that different concentrations among animals may be the result of different overall concentrations of IgM in the serum rather than different concentrations of natural antibodies that bind specifically to *Vibrio coralliilyticus* surface proteins. We considered this unlikely, however, because Dugovich et al. (2017) previously showed that 75% of the variation in *Vibrio coralliilyticus* reactive IgM was unexplained by total plasma IgM concentration. Thus, it is unlikely that natural IgM antibody concentrations in our study are directly related to total IgM concentrations in serum.

Humoral immunity can be affected by multiple pathways. In a previous study looking at Se status with regard to total immunoglobulin concentrations in cattle, it was shown that increased Se in the diet improved the humoral immune response to vaccination (Hall et al., 2011). In another study (Suganthu et al., 2019), sheep that received 1.5 mg/kg of organic Se supplement had an improved humoral immune response to *Peste des Petits Ruminants* vaccine, which is an
acute viral disease affecting goats and sheep. It has been shown that the immune status of a periparturient cow can influence passive immunity (Jezek et al., 2012). We tested for change in total IgM and natural antibody (*Vibrio coralliilyticus* specific IgM) concentrations with Se status in this study. A possible explanation for our results could be that the dosage of Se yeast given to the cattle was too low or too high, as a study done by Urban et al. (1986) found that phagocytic and bactericidal potential increased with dietary Se supplementation, but that at high concentrations there was a negative or no effect. The number of days that cattle were supplemented with Se-yeast is also an important factor in looking at humoral immunity. Additionally, supplementation time can affect production of selenotrisulfides, which can alter the amount of IgM secreted (Stabel et al., 1991).

Supplementing with organic Se-yeast during pregnancy, which is similar to feeding Se-enriched forage, may increase overall immune health. Feeding organic Se, i.e., that contains organically bound Se in the form of selenocysteine and selenomethionine, has been shown to be the most effective Se-supplementation method (Baulez et al., 2011). Knowing that Se has an effect on improving humoral immunity led us to ask the question as to when is the best time during pregnancy to supplement beef cows with organic Se to improve cattle health.

Selenium’s main role in immunity is through its incorporation into selenoproteins. Selenoproteins function as antioxidants, which help to break down peroxides that can cause damage to DNA and tissue. For example, certain kinds of selenoproteins are crucial for regenerating reduced forms of thioredoxin to maintain balanced concentrations of oxidized/reduced molecules within cells. Selenium concentrations in immune cells can affect the
oxidative burst processes in both phagocytic and nonphagocytic cells, meaning if there is Se deficiency, cells exhibit reduced oxidative burst (Baker et al., 1998). Additionally, a report shows that H-PGDS, an enzyme in macrophages that catalyzes the reaction of PGH₂ to PGD₂ and H-PGD₂ is regulated by the redox status of macrophages, and is dependent on Se concentrations (Gandhi et al., 2011). Both selenite and selenomethionine can also be metabolized into methylated Se compounds that have cancer chemo preventive effects (Huang et al., 2012).

In cattle, the main source of Se comes from grazing on grasses and hay produced from the forage grasses. If cattle reside in an area where Se is scarce in the soil, they are susceptible to Se deficiency (Hall et al., 2009; Lemly, 1997). Selenium deficiency can cause damage to skeletal muscles (white muscle disease, also known as nutritional myodegeneration) and cause pregnant cattle to have premature and weak calves (LeBlanc et al., 2004). Suboptimal Se intake also is associated with diseases such as mastitis and foot rot (Hall et al., 2009; Koller et al., 1983). Previous studies have also shown that supplementing with Se during late gestation can improve antioxidant function and relieve oxidative stress that occurs during early lactation (Gong & Xiao, 2018).

Because humoral immunity can be affected in multiple pathways, we will also test for differences in antigen-specific antibody responses. The cows received booster vaccinations during pregnancy so we can run titers on serum collected at baseline and at parturition after booster vaccination (e.g., to BVD) to test antigen-specific antibody responses. This will inform us whether IgG production to a specific antigen is affected with Se treatment. We will also evaluate nonspecific humoral immunity using a complement mediated bacterial killing assay to
*Vibrio coralliilyticus*. It is possible that improved Se status enhances complement-mediated killing of bacteria in plasma and that Se supplementation works via nonspecific humoral immunity.

Future studies to investigate how supranutritional Se supplementation with organic Se yeast affects catabolism of antibodies in calves might be interesting as concentrations will decrease until antibodies are actively made by responding to natural infections or vaccination. It has also been shown that Se-yeast supplementation alters erythrocyte GSH-Px and GSH concentrations one week postpartum (Gong et al., 2018). Looking into these concentrations may help us understand how supranational Se supplementation during pregnancy alters antioxidant status. Finally, as stated above, running titers on serum collected at baseline and at parturition from cows in each treatment group after booster vaccinations to test antigen-specific antibody responses will help us understand antigen-specific IgG antibody responses.

**CONCLUSIONS**

These findings have shown us how supplementing organic Se-yeast during pregnancy does not have an overall effect on IgM concentrations in beef cows at parturition. We do not provide evidence for affecting cow immunity by this pathway. This leaves room to explore how supplementing organic Se-yeast affects immunity by other mechanisms to improve cow health.
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