Chemical Analysis of Selenium and Copper Levels in Virginia Cattle Herds

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Abstract

Copper and selenium play an important role in maintaining a healthy cattle herd. Both of these minerals work with enzymes to reduce peroxides that could potential cause tissue damage. Random samples of blood, serum, and liver were taken from various cattle herds across Virginia. Serum was used to determine the copper concentrations, while blood and liver samples were used to determine selenium concentrations. The objective was to determine whether herds across Virginia had deficient, adequate, or toxic levels of copper and selenium. The various samples were either diluted with or dissolved in acid, while the concentrations were determined by using the Atomic Absorption Spectrophotometer. Both sets of cattle tested for copper were deficient. The first set of selenium samples was slightly deficient and normal, but the second set of selenium samples generally had levels that were reaching toxic levels. Based on the results, the trend for copper levels to be deficient in herds across the state, while selenium concentrations appeared to be adequately supplemented or reaching toxic levels.

Introduction

Like all organisms, cattle must obtain minerals, vitamins, and other nutrients to ensure adequate health and production. Producers need a diet that will allow for both a high quality and high growth yield. It is recommended that the cattle obtain 455 g/kg of dry matter (DM) per day. Out of this 455g/kg DM, 163 g/kg should be crude protein and 182 g/kg of crude fiber. The remaining amount of dry matter should provide the cattle the necessary minerals such as calcium, magnesium, copper, selenium, sodium, and phosphorus, and vitamins such as vitamin E (Owen 1979, Table 7.4). Feeds used to meet these requirements consist primarily of a grain, hay, and a mineral supplement feed.

As already mentioned, copper is an essential mineral to the bovine diet. Copper works with cardiac enzymes called super oxide dismutase. Copper is located in a variety of compartments within the body, and adequate levels differ in each compartment. Some of the locations include the bovine serum, whole blood, and the liver. Copper levels can be elevated to toxic levels, which will adversely affect the physiological status of the animal and decrease its health status. In the liver, the adequate amount of copper is 25 to 100 ppm wet weight (Puls, 1994). In the bovine serum, an adequate level of copper falls between 0.60 and 1.50 ppm wet weight (Puls, 1994). The cattle producer should not let the level of copper drop below 5.0 ppm wet weight in the liver or 0.50 ppm wet weight in the bovine serum (Puls, 1994). Likewise, the cattle producer should monitor the copper levels so as not to let the copper levels rise above 250 ppm wet weight in the liver or 4.00 ppm in the bovine serum (Puls, 1994).

Copper deficiency is most common in young grazing cattle. Symptoms of this deficiency include increased abomasal ulcers, the inability of calves to suckle, lameness, and retained placenta (Puls, 1994). Copper deficiency is the primary cause of specific disease states in a number of mammalian species and it could also play a secondary role in modulating the effects of other disease processes (McMurray, 1980).When copper levels are low, the immune response to an intracellular pathogen may be impaired or hindered. This slowed immune response was shown by Silvia Cerone DVM, et al (1989) of the Department of Physiopathology, Veterinary Science, Buenos Aires, Argentina. Female Aberdeen Angus heifers received a diet containing molybdenum and sulphate for nine months to create a copper deficient animal. The animals were then immunized with live *S1119 Brucella abortus,* and then exposed to the pathogen. As a result of the copper deficiency, serum copper concentration and activity of cerulopasmin were decreased. The total levels of immunoglobulin G and total complement concentration in the serum were also impaired by the low levels of copper in the cows. Therefore, antibody to *Brucella abortus* and proliferation of Concanavalin A and other soluble antigens-stimulated peripheral blood mononuclear cells were significantly lower. These results proved that the immune response to an intracellular pathogen might be delayed by copper deficiency in the heifer. For treatment of copper deficiency, producers can give copper injections or provide a copper feed supplement (Puls, 1994).

While rare, copper toxicity is still a problem that can be caused by raised levels of dietary copper in milk replacer, which causes chronic toxicity in young calves. Signs of copper toxicity include depression, anorexia, abdominal discomfort, jaundice, decreased milk production, and hemolytic crisis. For treatment, producers can supplement a ration with ammonium molybdate and sodium sulphate, which will reduce copper uptake and slowly reduce copper levels in the liver. Cattle producers can also administer Penicillamine. However, this treatment is expensive and only effective prior to hemolytic crisis (Puls, 1994).

Selenium has been considered a toxic agent producing various disease conditions due to an accumulation of selenium in the forage (Rosenfield, 1964). However, selenium is now regarded an essential micronutrient found throughout animal tissue. The actual concentration of selenium is directly proportional to dietary intake. Selenium is a component of the cytosol enzyme known as glutathione peroxidase. This enzyme has a key role in intracellular oxidation-reduction reactions (Schering, 1988). Along with vitamin E, selenium prevents oxidative damage to cells. The selenium levels of cattle can be most easily measured by an analysis of whole blood for either selenium or glutathione peroxidase (Schering, 1988).

As with copper, cattle suffer from selenium toxicity and deficiency. For a cow to remain healthy, adequate levels of selenium must be maintained. The following concentrations are considered adequate for cattle: liver samples between 0.25-0.50 ppm, serum samples between 0.08-0.30 ppm, and blood samples between 0.20-1.20 ppm wet weight (Puls, 1994). In order to be considered deficient, selenium levels must be below 0.17 ppm wet weight in the liver, or 0.08 ppm wet weight in the blood (Puls, 1994). Likewise, cattle selenium levels should be considered toxic if concentrations were to rise above 1.25 ppm wet weight in the liver, 3.50 ppm wet weight in the serum, or 14.00 ppm wet weight in the blood (Puls, 1994).

In Virginia, selenium deficiency is much more common than selenium toxicity. Symptoms of selenium deficiency include diarrhea, muscle stiffness, and sudden death due to cardiac failure, with no prior signs of sickness, and can cause white muscle disease. Selenium deficiency has also been shown to inhibit resistance to microbial and viral infections, neutrophil function, and proliferation of T and B lymphocytes (Swecker, 1990). In addition, with the changes associated with selenium deficiency, they may alter platelet function in vivo and thus play a role in the increased incidence of atherosclerosis (Hampel et al, 1989). At the school of Pharmaceutical Sciences in Tokyo, Japan, S. Hara et al (1989) tested the effect of selenium deficiency on oxidative stress-induced 8-isoprostane formation by bovine arterial endothelial cells. They first measured by enzyme immunoassay the level of 8-Iso produced by cultured bovine arterial endothelial cells (BAEC) exposed to oxidative stress. The scientists examined the enzyme 8-Iso formation in a selenium deficient media for a week. Glutathione peroxidase is an enzyme that protects cells against peroxidation and controls concentrations of peroxides. They found that selenium deficiency increased 8-Iso formation and that it increased oxidative stress. To treat selenium deficiency, producers may use injectable selenium at 0.13 mg Se/kg body weight. This will maintain the body reserves for 30-60 days (Puls, 1994).

Bovine blood and liver samples were obtained to measure the mineral concentration in the Atomic Absorption Spectrophotmetry. In an atomic absorption analysis the element being analyzed must be reduced to the elemental state, vaporized, and imposed in the beam of radiation from the source (Skoog, 1997). The basic principles of atomic absorption spectroscopy can be expressed by three simple statements. First all atoms absorb light. Second, the wavelength at which light is absorbed is specific for a particular chemical element. Third, the amount of light absorbed is proportional to the concentration of absorbing atoms. Essentially, quantitative analysis by atomic absorption spectroscopy is a matter of converting samples and standards into solutions, and then comparing the instrumental responses of standards and samples. These comparative responses are then used to establish accurate concentration values for the element of interest.

The purpose of this toxicity analysis was to determine if cattle diets located on Commonwealth of Virginia farms were providing adequate amounts of copper and selenium to cattle. After determining the amounts of copper, statistical tests were run to determine if there was any statistical difference between the mineral concentrations. The hypothesis for this research study was that differing diets would produce differing levels of the trace minerals selenium and copper in the bovine serum. The null hypothesis was that the selenium and copper levels would be identical in both sets of bovine serum. Because random samples were collected, the breed and diets of the cattle were unknown. The independent and the dependent variables were the differing diets of cattle and the concentrations of copper and selenium measured in the cattle, respectively. Copper and selenium are very important to the health of the cattle. It is important to establish a feeding regimen that provides cattle with appropriate levels of selenium, copper, and other essential nutrients.

Methods and Materials

All of our samples were shipped to the laboratory from various farms located around the Commonwealth of Virginia. The liver samples were delivered on ice and they were refrigerated

at four degrees Celsius. The blood samples were delivered in 5.0 mL tubes. The samples were either heparinized or mixed with EDTA to prevent clotting.

The first step is to measure the amount of copper in two sets of bovine serum. The blood serum samples were randomly divided into two groups for future evaluation. For sample preparation, all beakers and glassware were cleaned with acid to sterilize the glassware. Each 1 mL serum sample was then diluted with 0.5% nitric acid to a total volume of at least 2.5 mL.

For analyzing copper levels, the atomic absorption spectrophotometer was set to use the flame method. A copper hollow cathode lamp was used. This lamp source was set at a wavelength of 324.8 nm and the slit width was set at 0.5 nm. For this analysis, there was no background correction. The Atomic Absorption Spectrophotometer was set to "PROMT" for its measurement mode, "Concentration" as its calibration mode, and "Absorbance" as its instrument mode. For this particular analysis, the calibration algorithm was the New Rational algorithm. The bulk standard concentration was set at 6.0 ppm, and the standards were 1.2 ppm, 2.4 ppm, 3.6 ppm, 4.8 ppm, and 6.0 ppm. The following setting were used for proper analysis of the samples: three second measurements, eight second pre-read delay, 6.0 mA lamp current, and the lamp was set in the "third lamp" position.

For operation of the atomic absorption spectrophotometer, it was set up with the air/acetylene burner, and the SIPS unit. The serum weights and the total sample weights were then entered into the appropriate computer program. To optimize the lamp, the flame was ignited and then the flame was extinguished. The flame was reignited and started and the pump tube was inserted into each sample.

The second aspect of the research study was to measure the selenium concentrations in the cattle. A different group of cattle was used for selenium evaluation. Samples were again divided into two sets for measuring selenium. One set had a total of ten cattle, and the other set had a total of nine cattle. For sample preparation, all glassware was cleaned with concentrated nitric acid in order to sterilize the equipment. Then for the blood sample, the tube of blood was vortexed for about one minute or until the sample looked homogeneous. A 50 mL beaker was tared, and 1 mL of whole blood was measured using a 100-1000 uL eppendorf pipette. For the liver sample, one gram of liver tissue was digested for analysis using approximately 12 mL of a 4:1 nitric:perchloric mixture. Then with the hood fan and water on, the samples were heated on a hot plate at setting four (approximately 125-200^{O} C) until a dense white smoke formed. At that point, the hot plate was turned down to setting three. Once the solution had evaporated to about 1 mL, the sample was poured into a test tube that was designated for selenium evaluation. After pouring, the beaker was rinsed four times with twenty percent hydrochloric acid, pouring the residue into the tube, to the bottom of the white circle, to reach the final volume of 20 mL. Next, the sample was inverted three times, and analyzed one hour later. The selenium samples were then placed in the atomic absorption spectrophotometer for analysis, using the Vapor Generation Accessory (VGA) method. This method uses a flame and a glass tube to evaluate selenium concentrations. The solution passes into the glass tube, which is surrounded by the flame, and then the selenium level is measured.

Two-tailed t-tests were used to evaluate results. This test compares the variance and means of two groups. Statistical test were run for both minerals, comparing the two groups of copper and the two groups of selenium. The alpha level for this study was set a 0.05. A t-test calculator provided at http://www.graphpad.com was used to calculate all t-values. Then with the determined t-values and the degrees of freedom, and the use of a provided table, the students determined statistical difference. If the calculated t-value was equal or higher than the t-value on the statistical table, then there was statistical significance between the two compared groups.

Results

At the conclusion of this research study, the copper and selenium concentrations for each set of cattle were tested for statistical difference. The two sets of cattle measured for copper were compared first. Set A had an average copper concentration of 0.595 ppm wet weight and a standard deviation of 0.149. Set B had an average copper concentration of 0.456 ppm wet weight, and a standard deviation of 0.101. The t-value was calculated to be 1.54, which indicated no statistical difference between the two compared sets. Therefore, there was no statistical difference between the two sets of cattle measured for copper concentrations. Please see Figures 1 and 3.

Two sets of cattle were also tested for selenium concentrations in their blood and liver tissue. For the first set of cattle, liver and blood samples were analyzed. Set A had an average selenium blood concentration of 205 parts per billion, and an average selenium liver concentration of 135 parts per billion. For the second group, only liver samples were analyzed. Set B had an average selenium liver concentration of 2800 parts per billion. The first group generally had normal levels of selenium, which is 200-300 parts per billion. The second group had higher concentrations of selenium, some of which were at or near the toxic level, which is over 500 parts per billion. These two groups were not compared to each other because they contained different combinations of samples (i.e., blood and liver). Please see Figures 2 and 4.

Discussion

The purpose of the research study was to determine if cattle herds across the Commonwealth of Virginia had deficient, adequate, or toxic concentrations of copper and selenium. The null hypothesis was that the levels of copper or selenium would be the same between each set of cattle respectively. The hypothesis for this research study was that different diets would produce differing levels of the trace minerals selenium and copper in the bovine serum, blood, and liver tissues.

The t-test showed that there was no statistical difference in copper concentrations between Group A and Group B. Therefore, the null hypothesis was supported. Adequate levels of copper are between 0.60 and 1.50 parts per million (Puls, 1994). However, all cattle sampled had copper concentrations below the minimum value. This finding was not surprising because copper deficiency is a common disease in cattle. It is extremely important to quickly provide treatment to these deficient animals. In the case of copper deficiency, the cattle producer can either inject copper directly into the cattle, or supplement copper into the cattle's feeding regimen.

Adequate levels of selenium in the blood are between 200 and 1200 parts per billion (Puls, 1994). In Set A, the blood samples had an average selenium level that fell within this range, and therefore were considered adequate. Adequate levels of selenium in the liver are between 250 and 500 parts per billion (Puls, 1994). However, the liver samples in Set A were below this range, and therefore considered deficient. Set B had an average liver concentration above the acceptable range, which could lead to selenium toxicosis. For treatment, the cattle producer should reduce the selenium in the animal and the environment.

There are several improvements that would make the study more reliable. First, more samples should be evaluated to get a better analysis of the Virginia herds. More serum, blood, and liver samples would make the study much more accurate and reliable. Second, it would be beneficial to know the animal's history (i.e., health, diet, and age of cattle). If the diets were available, it would be possible to speculate the cause of the deficiency or toxicosis of copper or selenium in the cattle, leading to a more rapid treatment of the cattle.

Conclusion

In conclusion, minerals are important to all mammals. Specifically, copper and selenium are essential to the health of the cattle. It is critical for producers to establish a feeding regimen that provides cattle with appropriate levels of selenium, copper, and other essential nutrients. For animals that are suspected deficient or toxic, it is necessary to be treated quickly, evaluate herd nutrition, and keep the disease from becoming chronic.

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Appendix:

Table1. Copper levels in cattle (Puls 1994).

Diets	Liver (ppm)	Kidney (ppm)	Serum (ppm)
Deficient	0.50-10.0	1.0-5.0	0.02-1.00
Marginal	5.0-25.0	3.0-5.5	0.50-1.20
Adequate	25-100	4.0-6.0	0.60-1.50
High	200-550	5.0-7.0	2.50-4.00
Toxic	250-800	10-122	4.00-11.0

Table 2. Selenium levels in cattle (Puls 1994).

Diets	Liver (ppm)	Kidney (ppm)	Serum (ppm)	Blood (ppm)
Deficient	0.02-0.17	0.18-0.40	0.002-0.025	0.004-0.080
Marginal	0.12-0.25	0.40-1.00	0.030-0.060	0.060-0.160
Adequate	0.25-0.50	1.00-1.50	0.080-0.300	0.200-1.200
High	0.75-1.25	2.00-2.50	2.500-3.500	10.00-14.00
Toxic	1.25-7.00	2.50-5.00	3.500-4.100	14.00-16.40

Table 3. Specification of optimum nutrient levels in diets for cows (Owens, 1991).

ME (MJ/kg DM)	**Dairy cows in milk**	**Suckling beef cattle**
Crude Protein (g/kg DM)	140	130
Crude Fiber (g/kg DM)	160-200	170-300
Calcium (g/kg DM)	5.0	5.0
Phosphorus (g/kg DM)	4.0	4.0
Magnesium (g/kg DM)	2.0	2.0
Sodium (g/kg DM)	1.5	1.5
Vitamin A (ug/kg DM)	1500	3500
Vitamin D (ug/kg DM)	15	15

Figure 1. Mean Copper Concentrations.

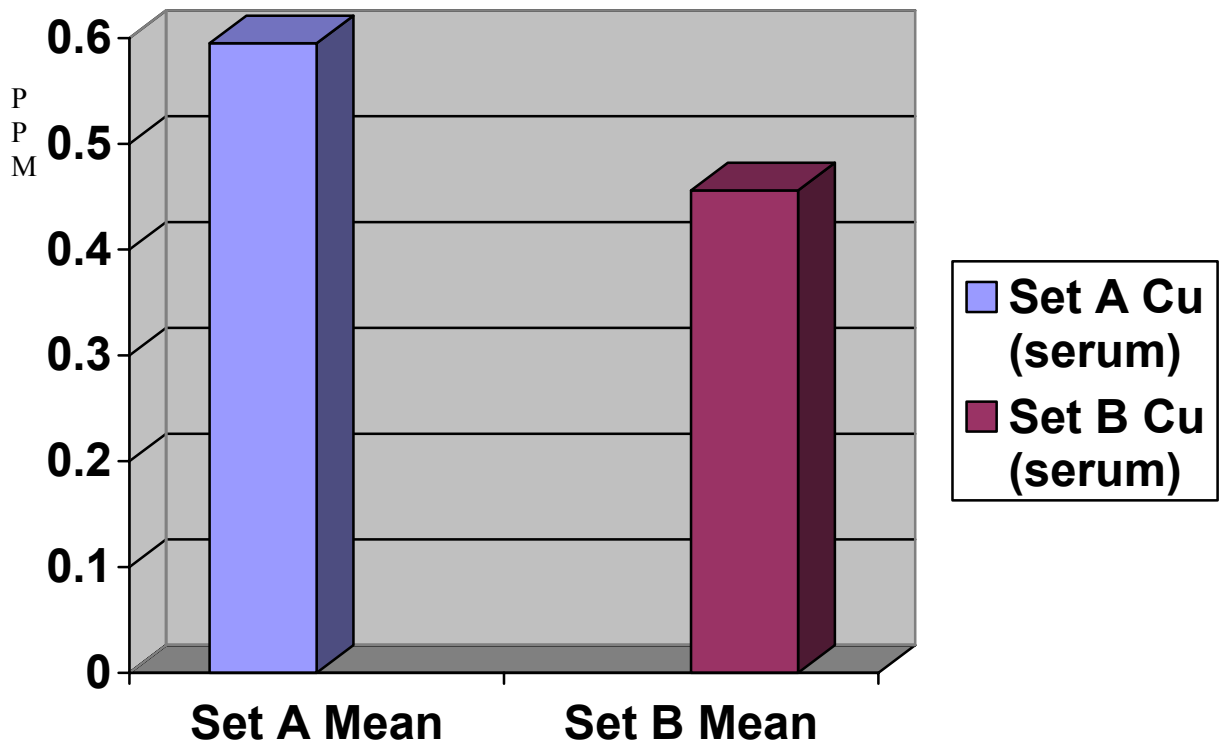

Figure 2. Mean Selenium Concentrations.

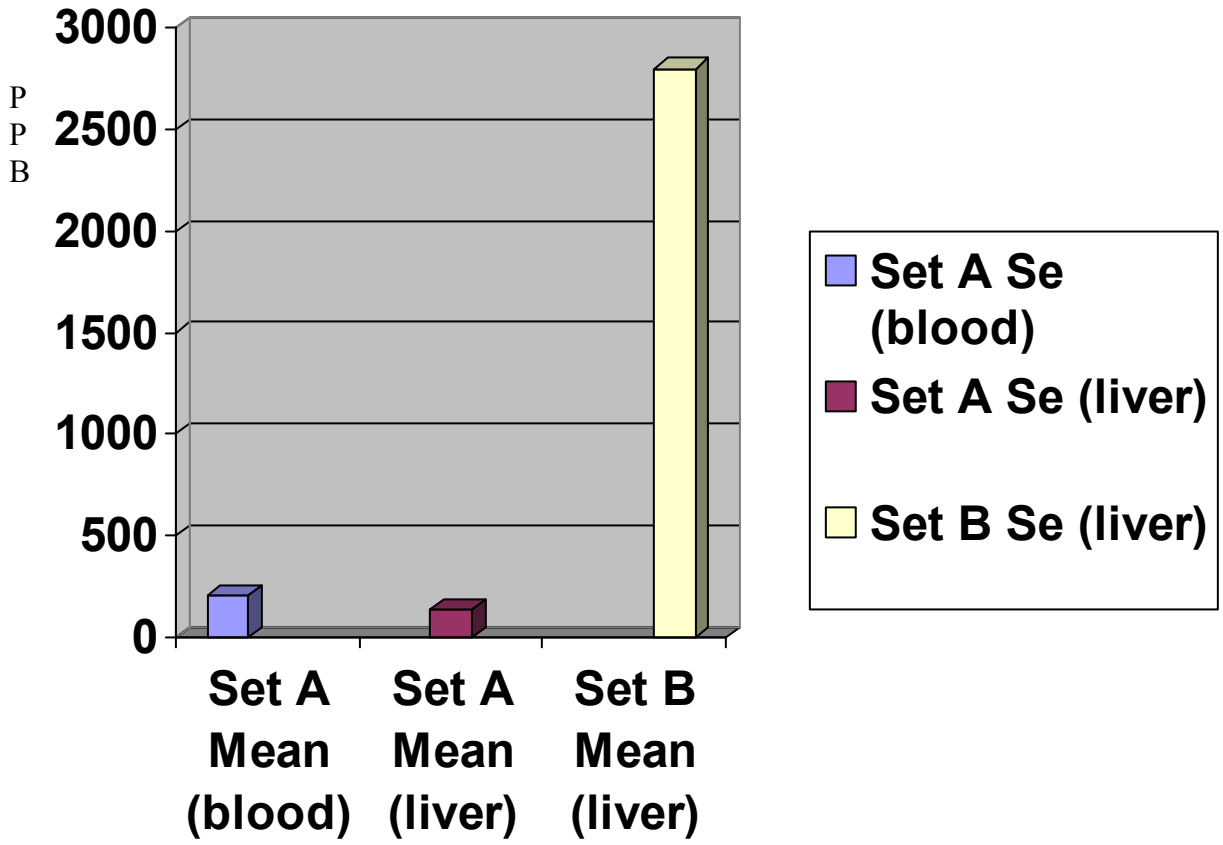

Figure 3. Individual Copper Concentrations.

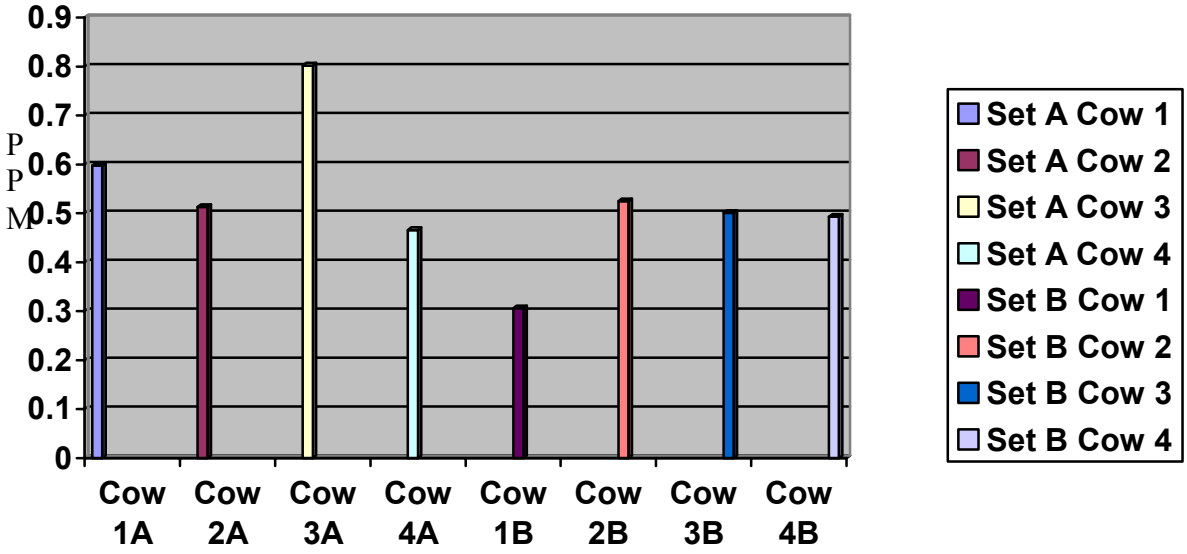

Figure 4. Individual Selenium Concentrations.