

The Effects of Organic Selenium on Rumen Volatile Fatty Acid Production in Continuous Culture

Dean Feasenhiser

ABSTRACT

Selenium is a potentially toxic trace mineral that is known to be a requirement for ruminant animals. Selenium is most easily absorbed in the form of selenomethionine, but is commonly supplied in the form of sodium selenite. The goal of this experiment was to recreate the rumen environment to determine the effects of selenomethionine at levels of 1.1 and 2.5 ppm. Rumen effects were measured by changes in volatile fatty acid (VFA) concentrations using gas chromatography. The study concluded that there were no significant effects in changing from a normal level (1.1 ppm) of selenomethionine to the higher level (2.5 ppm). However, a trend was detected that showed minor decreases in most VFA levels at the high level of selenomethionine.

Keywords: *rumen, ruminant, selenomethionine, selenium, volatile fatty acid.*

INTRODUCTION

Selenium was first identified as potentially toxic when it was discovered in the nineteenth century by the Swedish chemist Jons Jakob Berzelius. However, in the 20th century selenium was shown to be beneficial to organisms when present in relatively small amounts. Selenium also controls free radicals by enabling glutathione peroxidase. Livestock nutritional deficiencies in selenium have been shown to cause a condition known as white muscle disease. The recommended level of selenium intake is 0.2 to 2 ppm for cattle (Seman et. al., 1985). In toxic amounts, selenium can cause lameness and abscesses on the tongue, as well as red blood cell fragility.

Hidiroglou and Lessard (1976) showed that an increase in the amount of sodium selenite added to the diet of sheep had an effect on the volatile fatty acid (VFA) content of the rumen in that it increased the concentration of isovaleric acid. Vitamin E, which has some of the same functions as selenium, was also shown to have an impact on VFA concentrations by the Hidiroglou and Lessard study in 1976 and by Naziroglu et. al. (2001). In these studies, the addition of vitamin E decreased the concentration of butyric acid and increased the concentration of acetic acid.

VFAs are short chain carbon compounds that are produced by the microbial flora of the rumen. The ruminant animal is dependent on these fatty acids for most of its energy needs. A reduction of these fatty acids could cause an energy deficiency, as well as signal a change in the makeup of the microbial population. Several VFAs have been shown to have specific effects on the ecology of the rumen. Butyric acid was reported by Mroz et. al. (2000) to have a positive effect on the digestion of certain amino acids. Acetic acid has been shown to be a major energy supplier for the animal (Peters et. al., 1992). Propionic acid serves as a precursor to glucose and

provides a more efficient source of energy. A decrease in isovaleric acid has been shown to be associated with an increased use of nitrogen by growing bacteria (Petit and Veira, 1994). Specifically, isovaleric acid is known to act as a carbon skeleton for the production of leucine by bacteria in the rumen (Hidiroglou and Lessard, 1976). VFA levels can be utilized as a general measure of microbial activity.

Prior studies have generally focused on looking at supplementing diets with sodium selenite (Seman et. al., 1985 and Hidiroglou and Lessard, 1976). However, selenium has been found to be more easily absorbed in the form of selenomethionine (Awadah et. al., 1998), which is a methionine with the sulfur atom replaced by a selenium atom.

The purpose of this study was to examine the effects of selenomethionine on rumen VFA concentrations. If toxic concentrations of selenium cause a negative effect to microbial populations, this could have implications on the ruminant's recovery period following periods of selenium toxicity. While the rumen microbe populations would eventually return to normal, there are products available to inoculate the rumen that may speed the recovery of the rumen micro-flora. These could be administered to animals to shorten the period of reduced gains.

MATERIALS AND METHODS

This experiment used a chemostat to simulate the rumen environment. Rumen fluid was used to inoculate the cultures, and gas chromatography was used to determine the concentrations of VFAs.

COLLECTION OF RUMEN FLUID

Rumen fluid was collected from slaughtered beef

cattle. This was done at Krehbiel's Specialty Meats in McPherson, Kansas with the aid of William Hutcheson, DVM. The fluid was collected by making an incision in the rumen and removing material and fluid from the rumen with a cup. The fluid was then placed in a thermos for transport to the lab. The fluid was then filtered through cheesecloth (Griswold et al., 1996).

CONTINUOUS CULTURE SYSTEM

The chemostat used was a Virtis Omni Culture Plus. The containment vessel had a working capacity of 600 ml. The fluid addition pump was the Masterflex LS model 7523-50.

A buffer was required to simulate the flow of nutrients, water, and saliva into the rumen. The buffer solution of Wampler et al. (1998) was used for this study. This fluid includes 292 mg/L K_2HPO_4 , 240 mg/L KH_2PO_4 , 480 mg/L $(NH_4)_2SO_4$, 480 mg/L NaCl, 100 mg/L $MgSO_4 \cdot 7H_2O$, 64 mg/L $CaCl_2 \cdot 2H_2O$, 4,000 mg/L Na_2CO_3 , 600 mg/L Cysteine Hydrochloride, 1,000 mg/L Trypticase, 1 g/L of a glucose, and 500 mg/L yeast extract. This fluid was added at a rate of 1.2 ml per minute.

To maintain anaerobic conditions, nitrogen gas was pumped in continuously. Nitrogen was used rather than CO_2 to prevent possible pH decrease due to carbonation (Hoover et al., 1976). A peristaltic pump supplied the buffer solution to the experimental container. Also required for the continuous culture system were 5 N HCl and 5 N NaOH (Hoover et al., 1984). These were used to maintain the pH, and were also automatically infused by the chemostat system. The system pH was set at the buffer solution pH of 6.7. The fluid in the experimental container was agitated at a constant 50 rpm. Temperature was maintained at 39° C (Griswold et al., 2003).

To maintain proper regulation of pH and microbial concentrations, the liquid dilution rate was 0.12 of the total volume per hour. (Griswold et al., 2003) This dilution rate resulted in 2.88 cycles of fluid per day. The test was run for 10 days with a volume of 0.6 liters; each test required 1.73 L of buffer solution per day plus 0.6 liters to fill the chemostat for a total of 17.9 L.

Each of the two tests was divided into two 5-day trials with selenium levels of 1.1 ppm for the first trial and 2.5 ppm for the second trial. Two days were allowed for the system to come to equilibrium before sampling occurred. This allowed another three days for sampling purposes. Samples for fatty acid analysis were taken daily for three days.

SELENIUM QUANTITIES

The selenium levels were based on NRC recommendations of 0.2 to 2 ppm (Seamen et al. 1985). Selenomethionine was used as the selenium

source. The trials consisted of concentrations of 1.1 and 2.5 ppm.

VFA ANALYSIS

Samples for VFA analysis were taken using a vacuum bulb on the chemostat containment vessel. Samples were prepared by centrifuging a 5 ml sample of rumen fluid and 1 ml of a 25 percent metaphosphoric acid solution. The mixture was allowed to sit for 30 minutes and then centrifuged for ten minutes at 3,000 rpm according to the method described by Erwin et al. (1961). VFAs were analyzed by gas chromatography with a flame ionization detector at the Kansas State University Department of Animal Science nutrition laboratory. The cultures were further centrifuged for 15 minutes at 10,000 rpm. The column used was a 6' 1/4" column packed with GP 10% SP-1200 and 1% H_3PO_4 in 80/100 Chromosorb W AW from Supelco. The injection block and detector were maintained at 250° C and the column was maintained at 120° C. The nitrogen flow rate was 80 ml/m. Hydrogen flow to the flame jet of 20 ml/m with airflow to the chamber at 200 ml/minute and a combined flow set at 220 ml/m. Samples were analyzed in duplicate. Samples were analyzed for concentrations of acetate, valerate, isovalerate, butyrate, isobutyrate, and propionate. The gas chromatograph used was an HP 5890 with an attached auto-sampler. Integration of results was carried out by computer.

STATISTICAL ANALYSIS

Data was entered into Microsoft Excel and then transferred into SigmaStat for Windows Version 3.0.1 for statistical analysis. For each VFA, a two-way analysis of variance was performed on the mean of the two values obtained from each sample. The data was analyzed to detect any significance due to the level of selenium, differences between the two tests, and interactions between the selenium level and the tests. Also, the valerate results were analyzed using an all pairwise multiple comparison procedure using the Holm-Sidak method.

RESULTS

There were no significant differences in the concentrations of VFAs between the two levels of selenium (Table 1 and Figure 1). Valerate was the only VFA that showed a significant difference ($P < .05$) between the two tests performed (Table 2). There were no significant interactions between the test and the level of selenium.

Total VFA concentrations were compared against each other in the same manner of the other VFA concentrations. There were no significant differences (Tables 1 and 2, Figure 1).

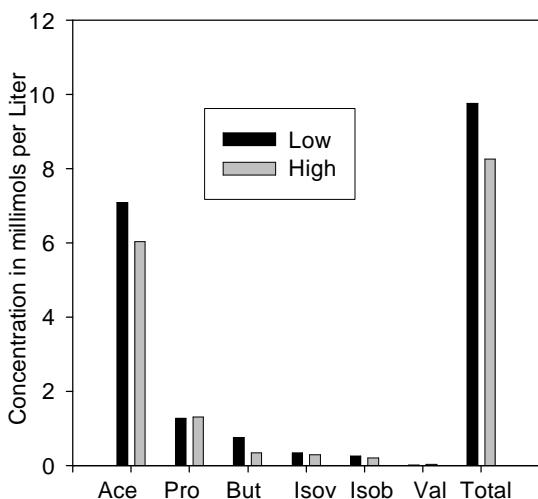
For valerate, significance was detected between

Table 1. VFA levels in millimols per liter for high and low selenium concentrations.

VFA	Low	High	P Value
Acetate	7.090	6.036	0.528
Propionate	1.277	1.308	0.940
Butyrate	0.761	0.355	0.125
Isovalerate	0.359	0.302	0.590
Isobutyrate	0.258	0.219	0.577
Valerate	0.020	0.042	0.168
Total	9.764	8.262	0.534

Table 2. VFA levels produced in the two tests expressed in millimols per liter.

VFA	Test 1	Test 2	P Value
Acetate	5.248	7.878	0.139
Propionate	1.190	1.395	0.630
Butyrate	0.467	0.649	0.464
Isovalerate	0.271	0.391	0.268
Isobutyrate	0.186	0.291	0.150
Valerate	0.010	0.052	0.019
Totals	7.371	10.656	0.193

**Figure 1.** VFA concentration between low and high concentrations of selenomethionine.

the two tests (Table 2). For the pairwise comparison, there was significance detected between runs 1 and 2 in the high level of selenium. The second test had significantly higher valerate concentrations. A significant difference was also detected within test 2 between the high and low levels of selenium. The high level of selenium produced a higher concentration of valerate in this test.

While no significant differences were observed, there appeared to be a decrease in the VFA concentrations at higher selenium levels. This was seen by looking at the mean concentrations in the

low and high levels of VFAs (Table 1 and Figure 1).

DISCUSSION

While not significant, the trend towards higher concentrations of VFAs with the lower levels of selenium suggests that a connection could be present between the two levels of selenomethionine studied and VFA production. It should be emphasized that this is a weak connection that should be explored further before being confirmed. This could be achieved by running the experiment for a longer period and taking more samples.

The lack of significance suggests that an increase in selenomethionine intake in cattle similar to that produced in the experiment would not have a detrimental effect on the rumen microflora. For the rancher, this would mean that an animal would, barring any toxicity reactions by the animal, continue to gain normally if exposed to this level of selenomethionine. The trend toward lower VFA levels suggests that an increase of selenomethionine beyond this point may have consequences for the VFA production of the rumen. This decrease could result in decreased energy supplied by the absorption of VFAs and therefore decreased weight gain by the animal.

While a significant difference in valerate levels was present in the second test, valerate is of less importance to the animal due to the low percentage of valerate as a total of the VFAs present.

ACKNOWLEDGEMENTS

I would like to thank the faculty of the natural science department for helping to make this project possible, with a special thanks to Professor Al Dutrow for being my research advisor. I would also like to thank Dr. Hutcheson and Krehbiel's Specialty Meats for supplying the rumen fluid, as well as the Kansas State University Animal Science Department for providing the gas chromatograph and expertise in sample analysis.

LITERATURE CITED

- Awadeh, F. T., R. L. Kincaid, and K. A. Johnson. 1998. Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves. *J. Anim. Sci.* 76:1204-1215.
- Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *J. Dairy Sci.* 44:1768-1770.
- Griswold, K. E., G. A. Apgar, J. Bouton, and J. L. Firkins. 2003. Effects of urea infusion and ruminal degradable protein concentrations on microbial growth, digestibility, and fermentation in continuous culture. *J. Anim. Sci.* 81:329-336.

- Griswold, K. E., W. H. Hoover, T. K. Miller, and W. V. Thayne. 1996. Effect of form of nitrogen on growth of ruminal microbes in continuous culture. *J. Anim. Sci.* 74:483-491.
- Hidroglou, M. and J. R. Lessard. 1976. The effect of selenium or vitamin E supplementation on Volatile fatty acid content of rumen liquor in sheep fed a purified diet. *Internat. J. Vit. Nutr. Res.* 46:458-463.
- Hoover, W. H., B. A. Cooker, and C. J. Siffen. 1976a. Effects of differential solid-liquid removal rates on protozoa numbers in continuous cultures of rumen contents. *J. Anim. Sci.* 43:528-534.
- Hoover, W. H., C. R. Kincaid, G. A. Varga, W. V. Thayne, and L. L. Junkins, Jr. 1984. Effects of solids and liquid flows on fermentation in continuous cultures. IV. pH and dilution rate. *J. Anim. Sci.* 58:692-699.
- Hoover, W. H., P. H. Knowlton, M. D. Stern, and C. J. Sniffen. 1976b. Effects of differential solid-liquid removal rates on fermentation parameters in continuous cultures of rumen contents. *J. Anim. Sci.* 43:535-542.
- Mroz, Z., A. W. Jongbloed, K. H. Partanen, K. Vreman, P. A. Kemme, and J. Kogut. 2000. The effects of calcium benzoate in diets with or without organic acids on dietary buffering capacity, apparent digestibility, retention of nutrients, and manure characteristics in swine. *J. Anim. Sci.* 78:2622-2632.
- Naziroglu, M., T. Güler, and A. Yüce. 2001. Effect of vitamin E on ruminal fermentation. *J. Vet. Med.* 49:251-255.
- Peters, J. P., R. Y. W. Shen, and J. A. Robinson. 1992. Disappearance of acetic acid from the bovine reticulorumen at basal and elevated concentrations of acetic acid. *J. Anim. Sci.* 70:1509-1517.
- Petit, H. V. and D. M. Veira. 1994. Digestion characteristics of beef steers fed silage and different levels of energy with or without protein supplementation. *J. Anim. Sci.* 72:3213-3220.
- Seman, D. H., N. Gay, K. A. Dawson, and J. A. Boling. 1985. Rumen bacteria response to different selenium concentrations in vitro. Progress report – Kentucky Agricultural Experiment Station 291:34-35.
- Wampler, Jennifer L., Scott A. Martin, and Gary M. Hill. 1998. Effects of laidlomycin propionate and monensin on glucose utilization and nutrient transport by *Streptococcus bovis* and *Selenomonas ruminantium*. *J. Anim. Sci.* 76:2730-2736.