Recreating a Ruminant Environment to Solve the Digestibility Factor of Plants For Wild Ruminant Animals: Mineral Oil Effects On Rumen Microbe Cultures

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Introduction

The ruminant gut has many different kinds of protist and bacterial microfauna that allow it to break down cellulose and plant matter. These microfauna are extremely important to the animal as these organisms provide extra nutrients from material that not all animals can utilize. Culturing rumen microfauna samples provides a researcher with a very difficult task. Since these types of microbes must be kept in an anaerobic environment, oxygen must not be allowed into the system or the microbes will die.

Experiment Goals

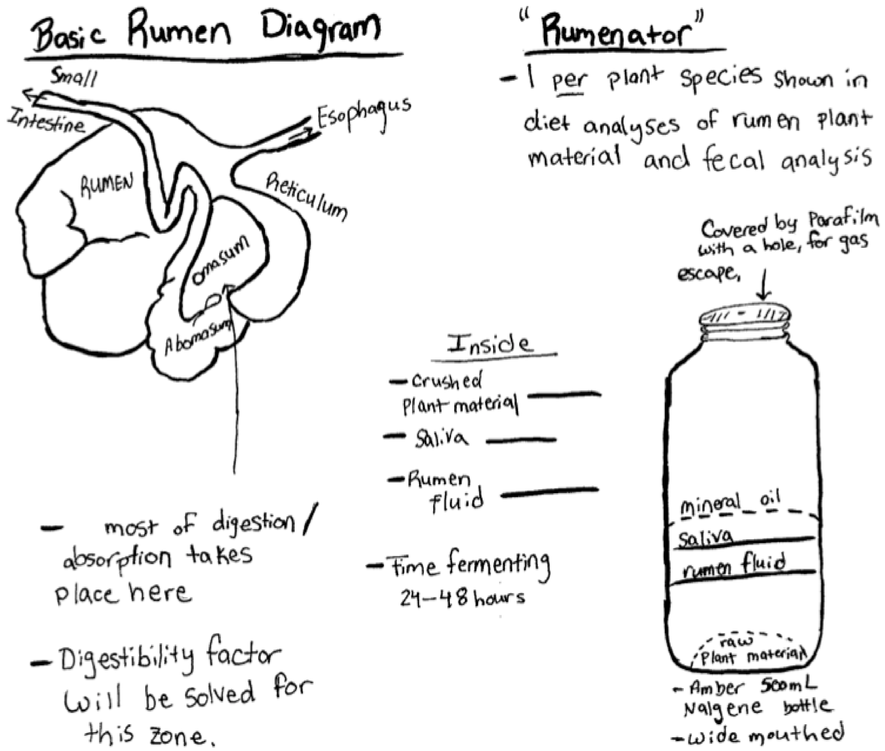
The initial goal was to test how microbe communities changed with different plants types. However, oxygen introduction due to the inefficiency of mineral oil became a problem. The goal was changed to think of a new protocol for the small scale rumens. This project focused more on keeping a rumen microfauna culture in the end rather than a large scale hypothesis.

Methods

Several different types of plants were tested as they fermented in rumen fluid taken from fistulated cattle from a cattle barn in St. Paul. After each collection, mineral oil was added to the collection to keep anaerobic for transportation. In the lab, fluid was taken from the bottom to put in culture and added to a bolus of plant material with a known mass. They were left to ferment in a water bath at 37.8°-40°C for 24 hours. Several trials were done with different plant types, noting differences in color, consistency, and smell. The boluses were pulled from the soup with cheesecloth and dried over 1-9 days. The amount of mass lost was used to judge how well the fermentation worked in the "rumenators".

Protist work was also done in order to detect levels of holotrichs, ciliates, and entodiniomorphids under a microscope with fresh sealed slide samples. However, the knowledge for certain identifications were lacking and the protocol was not maintained completely devoid of oxygen. This was a requirement that was not met and made the microfauna samples statistically and informatively not useful.

Figure 1. a) A basic diagram of a rumen to show the proportion of the stomach system that is dedicated to the fermenting rumen chamber. b) the proposed design of the small scale "rumenator".



Results

As shown in the tables below, as the boluses were set to dry, the masses were shown to still be above what was put into the "rumenators". The boluses dried for weeks and still the masses did not show real loss of matter.

When redrawing the protocol design, beer brewing air locks were added onto the top to introduce a fully anaerobic environment that was not as easy to disturb as mineral oil.

Table 1. These tables show the results of the trials using different stocks to test the cultures and the efficiency of fermentation/digestion of the plant boluses. These trials occurred on several dates and the boluses were reweighed as the experiment went on in attempts to fully dry the boluses.

Trial Date	Bollus Mass Added (barn feed)	Rumen Fluid Added	Mineral Oil Added	Extra added	Mass after	Total Material Lost
7/22/13	15 g	30ml	20 ml	20 ml DH_2O	10.54 g	4.46g
7/24/13	11g	30ml	0ml	20ml DH_2O	10.90g	0.10g

Trial Date	Bollus Mass Added (silver maple 1)	Rumen Fluid Added	Mineral Oil Added	Extra Added	Mass after	Total Material Lost
7/30/13	10.02g	30ml *old stock (1)	10ml	0ml	8/2/13 22.26g 8/5/13 18.04g 8/10/13 15.98g 8/26/13 15.50g	8/2/13 +12.24g 8/5/13 +8.02g 8/10/13 +5.96g 8/26/13 +5.48g
7/30/13	10.06g	30ml *new stock (2)	10ml	0ml	8/2/13 16.25g 8/5/13 13.08g 8/10/13 12.03g 8/26/13 11.99g	8/2/13 +6.19g 8/5/13 +3.02 8/10/13 +1.97g 8/26/13 +1.93g

Table 2. This table shows moreresults of the trials using different stocks to test the cultures and the efficiency of fermentation/digestion of the plant boluses. These trials occurred on several dates and the boluses were reweighed as the experiment went on in attempts to fully dry the boluses.

Trial Date	Bollus Mass Added (silver maple 2)	Rumen Fluid Added	Mineral Oil Added	Extra Added	Mass after	Total Material Lost
8/9/13	10.03g	30ml *oldest stock (1)	10ml?	0ml	8/9/13 13.98g 8/26/13 13.80g	8/9/13 +3.95g 8/26/13 +3.77g
8/9/13	10.06g	30ml *old stock (2)	10ml?	0ml	8/9/13 11.32g 8/26/13 11.13g	8/9/13 +1.26g 8/26/13 +1.07g
8/9/13	10.01g	30ml *new stock (3)	10ml?	0ml	8/9/13 9.44g 8/26/13 9.36g	8/9/13 0.57g 8/26/13 0.65g

Discussion

This project became more about refining protocol than about collecting hard data, since collecting data became quite difficult when the system kept failing. It was noticed that, upon trying to draw out cultures of the protists, the mineral oil failed to be an anaerobic barrier for the microbes in the fermenting zone. Thus it was determined that the protocol needed to have a means of keeping oxygen out that performed as a better oxygen barrier than mineral oil. In addition, the boluses became very hard to fully dry out since the mineral oil added mass and did not air dry.

This protocol is going to need further testing in order to become a fully usable field method to test different plant matter against wild caught ruminant rumen fluid. Often times, the rumen fluid is gathered from freshly deceased individuals during a necropsy. The protocol developed during this experiment would be of great use to researchers meaning to do this type of testing. This small scale rumen system would help in determining if the microbe community is changing too quickly for species, such as *Alces alces*, to adapt. In conjunction with many wildlife researchers and programs, these small scale rumens could provide valuable information for preserving the species as well as what is afflicting them internally and nutritionally.

References

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