Report to The Stapledon Memorial Trust

To develop in-vitro methodology to predict methane emissions from ruminant livestock ingesting green forages

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Background

The objective of the research carried out during the Fellowship period was to develop in-vitro gas production procedures for green forages ingested by ruminants. Such methods would allow reliable evaluation of nutritive value and methane production of shrubs and foliages. Current concerns about food security and climate change suggest that future constraints relating to world agriculture will be wide-ranging and severe. Compared with the present, more human food will need to be produced in a sustainable manner on less available land, and using lower fossil fuel inputs with reduced emissions of greenhouse gases. Furthermore, future agricultural systems should have positive impacts on biodiversity and the general health of the environment, and the welfare of farm livestock must not be compromised. As a consequence of this, livestock can be considered to continue to have an important role, but their dependence on grain as feed for efficient production will have to be drastically reduced. The challenge would be to develop efficient ruminant production systems based on feeding high-fibre (cellulosic) diets. In this present study, we tried to develop an easier method of estimating methane from the green forages, in vitro, which will be helpful in terms of development of sustainable livestock production.

Objective

The objective of the research carried out during the Fellowship period was to develop in-vitro gas production procedures for green forages ingested by ruminants. Such methods would allow reliable evaluation of nutritive value and methane production of shrubs and foliages. The period of research was 6 months. We had trials which needed to be re-validated by redesigning the trials to obtain good data from the experiments. Also, I carried our additional experiments in which we compared *in vitro* methane emission technology with the *in vivo* calorimeter studies at The James Hutton institute. An ongoing study in the institute will take time to get data for further analysis.

Samples comprised: (1) Straw- green frozen grass, and (2). Dried heather- Grass Hay, (freeze dried samples v/s oven dried)

Methodology followed

It is difficult to handle green material to put into syringes. The objective of this exercise was to check the different methods for estimating methane emission from the green material, which were as follows-

1. Coffee grinder with frozen plant material (-20^{o}C)

2. Also look at the difference between freeze dried and oven dried

Points that need to be kpt in the mind before the experiment:

1. Buffering capacity of incubation mixture

2. How to measure the CO2 production from the buffer or from the fermentation.

3. Ultimately want the product CH4 production/amount of the organic matter digested with a wide range of plant mixtures.

4. Use of tubes /Kjeldahl flask v/s syringes, using dry feeds and green samples. To check the difference between methane productions from freeze dried samples v/s oven dried.

Preparation of all the solutions for making buffer mixture

Apparatus/material: syringes glass and plastic, Kjeldhal flasks, tubes, small jars, rumen liquor, samples, distillation assembly, water bath, stand for flasks, stoppers, chemicals.

Preparation of the syringes

All the substrates should be milled using a 1 mm screen. Weigh 200 mg substrate into each (numbered syringes and record actual weight. Include the blank (i.e. rumen fluid/ buffer mixture on its own) at the beginning, in the middle of the set, at the end. A sample of hay can be milled and used as a control by including syringes with the hay at the beginning and at the end of each run. Samples should be done in the duplicates and triplicate. After the weighing is complete, grease the plungers with Vaseline, and place in incubator at 38^{o}C. This is normally done the day before the experiment running.

Preparation of the buffer

Measure distilled water, buffer solution, macro mineral solution, micromineral solution and resazurin solution into a round, flat bottomed, flask-warm to 38^{0}C then add reducing solution of NaS 9H2O, 1N NaOH and distilled water. Place in the small water bath on a magnetic stirrer, put a magnet in a flask and gently bubble CO2 through the solution until the blue colour turns to pink then clear - this means the buffer solution is now reduced. Raise the CO2 tube so that it will be above the level of the buffer/ rumen fluid mixture, but providing a stream of the CO2 tube so that o will be above the level of the buffer/ rumen fluid mixture,

but providing a stream of CO2 into the flask throughout the dispensing procedure (pH of the buffer should be pH 7-7.3)

Collect rumen fluid from the animals (normally 2) strain through gauze into warm beaker, the final ratio of buffer; rumen fluid should be 2:1 mix rumen fluid in a beaker and transfer to the flask with the buffer solution. Make sure the magnet is mixing properly during the whole process of dispensing the rumen fluid/ buffer mixture into the syringes. Add 30 ml to each syringe using the dispenser (do 2-3 30 ml amounts into a beaker at the beginning to be sure the dispenser is properly charged). Fill the syringe the open clip and gently push the plunger of the syringe so that all the gas is removed- record the level in each syringe and place in water bath.

Times of reading can be chosen to suit the time in the syringes. For forages 3hrs, 6hrs, 12hrs, 24hrs, 72hrs, and 96hrs are suitable, but to concentrate type substrate, it may be necessary to take more readings in the first 24hrs. It is advisable to gently mix each syringe 2-3 times during the first day; as well as each time a reading is taken.

The study

The prime objective of this study was to develop *in vitro* methodology to predict the methane emission from ruminant livestock ingesting green forages. As part of the Institute's research to examine the methane production from sheep and deer ingesting hill vegetation, it is recognized that there is a need to have simple methods for assessing methane production potential for the green material. A range of ingested plant species will be tested *in vivo* with sheep and deer in respiration chambers, but the *in vivo* option is not feasible for large numbers of different plant mixtures.

The first part of the study was to compare different methods of processing green plant material prior to measuring gas production and VFA composition during and after incubation with buffered rumen liquor. Processing methods are important because tannins and enzymes, such as polyphenol oxidase, which are present in many plants, may potentially interact with proteins and other compounds, altering ruminal fermentation characteristics, and hence modifying the nutritive value and methane production potential. Further method development will be required for the conduct of *in vitro* incubation procedures using rumen liquor collected from the animals used in the respiration chambers.

We also tried the same gas production method for fresh green grass by grinding it using a coffee grinder and mixer grinder. The results were not very satisfactory. A number of experiments fare needed for the further development of the method for green material. There was some gas production, but not in early hours of the experiment because of the particle size (the green material was not very fine as in in-vitro method we always use the fine ground sample for the experiments) The method can be further improved for the green material, which needs more experiments and a flexible time.

I was involved with the in-vitro gas production analysis from the syringes, tubes and flasks for different forage samples to see the methane production per unit of the organic matter degradation and also the volatile fatty acid production per unit of the organic matter degradation for the same samples. The first part of the study was to analyze the methane production per unit of degradation rate in different time intervals for forages that are frequently used by grazing animals in Scotland; in in-vitro trials by the use of syringes, tubes

and flasks, and also to compare it with *in vivo* methane production by calorimeter experiments for the same diets that were used for the *in vitro* method.

We used the simple Hohenheim gas production technique for gas production. We used a number of feeds for the analysis method. The method that we used for the total gas production was simple and we also used the gas for the methane analysis which needs to be calculated with the respiration chamber methane production.

Due to of the number of experiments, further work is needed on the data and it needs to be compared with the *in-vivo* method of gas productio. There is a need for one more experiment (by Dr. Mays) after completing the experiments. Only after getting all the data can we compile all the information together for the comparison.

The present results are compiled on the basis of the in-vitro methane production technique, i.e. the total gas production from the syringes from different forages. Due to the short period of time it is little difficult to put all the experiments and get the data analysis done in the six months, especially when both in-vivo and intro methods are going together for the comparison.

Conclusion and benefits:

This study has proved useful for the further development of animal production in a sustainable way. In relation to my personal career development, this advanced research work has contributed to improving my skills to deal with new animal research problems and also helped me for the developing recommendations for the communities while doing research and development work with them. I gained experience of some research techniques which will further help me to grow and present my work in the field of research and development.

Apart from receiving advanced research training, this experience has given me a great opportunity to explore other possible links between the host UK institute for the future partnerships and knowledge exchange between our countries.

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