Scientific report: Short-Term Scientific Mission (COST)

Sieglinde Debruyne – PhD student Ghent University

1. General information

COST Action:	FA1302
STSM title:	Acquiring rumen sampling skills in young ruminants and optimizing *in vivo* experimental set up.
Reference :	ECOST-STSM-FA1302-050514-044485
STSM dates:	From 05-05-2014 to 10-05-2014
Home institution:	Laboratory of Animal Nutrition and Quality, Melle, Belgium
Host institution:	Animal Nutrition Institute (EEZ), CSIC, Armilla-Granada, Spain
Host:	Dr David Yañez-ruíz (david.yanez@eez.csic.es)

2. Background information

Sieglinde Debruyne is an early-stage joint PhD researcher at Ghent University and the Institute for Agricultural and Fisheries Research (ILVO), Belgium. This short-term scientific mission (STSM) grant provided her with funding necessary to visit the Estación Experimental del Zaidín (EEZ), part of the CSIC in Granada, Spain. Both Belgium and Spain are countries participating in the Methagene COST action. Doctor David Yañez-Ruíz is the leader of the Small Ruminant Production research group at EEZ and the host of this STSM. He has agreed to guide Sieglinde in this five working days mission, where she has participated in the practical sampling work during the ongoing goat trial there.

3. Purpose of the STSM

The objective of this STSM was to allow the student, an early stage PhD researcher, to gain experience with the practice of rumen sampling by oral stomach tubing in small ruminants. The host institution has much experience with this basic technique. They have recently started a goat trial, during which many samplings will have to be performed on young and also adult goats. Additionally, the student was able to experience intensive handling of animals in trial and other sorts of sampling procedures, e.g. saliva and feces collection of live animals.

This STSM contributed to the scientific objectives of the METAGENE COST Action by optimizing an easily repeatable and satisfactory tool for rumen sampling in small ruminants, that can be used for large scale collection. Taking a representative sample of rumen fluid in methane reducing trials is crucial to investigate differences in ruminal fermentation parameters, perform subsequent microbial work and also *in vitro* methane production measurements. It is therefore

important that the sampling procedure to collect rumen fluid is standardized or at least comparable between experiments and preferably also between research groups. This will make interpreting results between different research groups easier and more trustworthy.

4. Experimental set up and performed activities

The goat trial going on at the moment of the mission is a trial in which the "early life programming" hypothesis is tested. This hypothesis states that phenomena occurring early in the life of an animal will have an (permanent) impact later in life. In this goat trial, they are testing the influence of early life supplementation of a yeast culture liquid and a different feeding protocol on the development of the rumen microbiome and methane emissions in goats during the productive life. This is done through treating the experimental group (12 mothers and 24 kids) with a dose of active liquid yeast culture twice daily, starting from birth until weaning. Next to this, half of these treated animals (12 kids) are separated from the mother, grouped together and fed with a bottle. Later in life, all the kids, including the control condition (no yeast supplementation, but also two feeding protocols), will be set up for an acidosis challenge (diet existing of 20:80 forage and concentrate, with a high degradable starch content).

From day one, Monday the 5th of May, I started my first sampling exercise in the stables of the EEZ. The goat kids were then between day 7 and day 10 of age. Sampling ages are days 7, 10, 13, 26, 42 and one month after and during the acidosis challenge. We performed sampling of the rumen via oral stomach tube, saliva collection, blood collection (for plasma), feces collection (when feasible) and recorded the weight of the kids. This was repeated on the following days until Friday, for the specific kids that had reached a specific sampling age. Next to this, I also helped with administrating the yeast culture solution to mothers and kids. This was done with a dosage gun for the mother, and a small syringe for the kids.

- **Rumen sampling**

First, all material (Figure 1) is placed on a clean table and tubes are connected before starting. Two persons are needed for a smooth course of the sampling procedure. One person holds the goat kid tight and holds the head in an upwards angle. The second person puts the short, hard plastic tube into the mouth of the kid and holds the head in such way that the tube does not fall out, without blocking the nostrils of the kid. Attention should be given to the place of the tongue. Then the person gently pushed in the flexible probing tube. The resistance you feel when pushing this over the esophagus should be low. If not, the tube could be misplaced and the introduction should be done again. The kid will protest heavily against the introduction of the tube, but once the tube has passed the throat, the protest is less. The tube should be pushed into the rumen until the mark on the tube reaches the mouth (estimated depth necessary for reaching the rumen). Then the vacuum pump must be turned on and the kid hold

as still as possible. The tube can be re-located if not enough fluid comes out, by breaking the vacuum (opening a premade hole in the tube) and then moving the tube deeper. After this, the vacuum must be closed again for sampling. When enough liquid is collected (generally in about 2 minutes), the vacuum is turned off and the tube is gently pulled back again. The hard plastic tube is taken away at the end. The rumen liquid (about 100 ml) was dispensed into two falcons (for a different type of analysis) and immediately stored on ice.

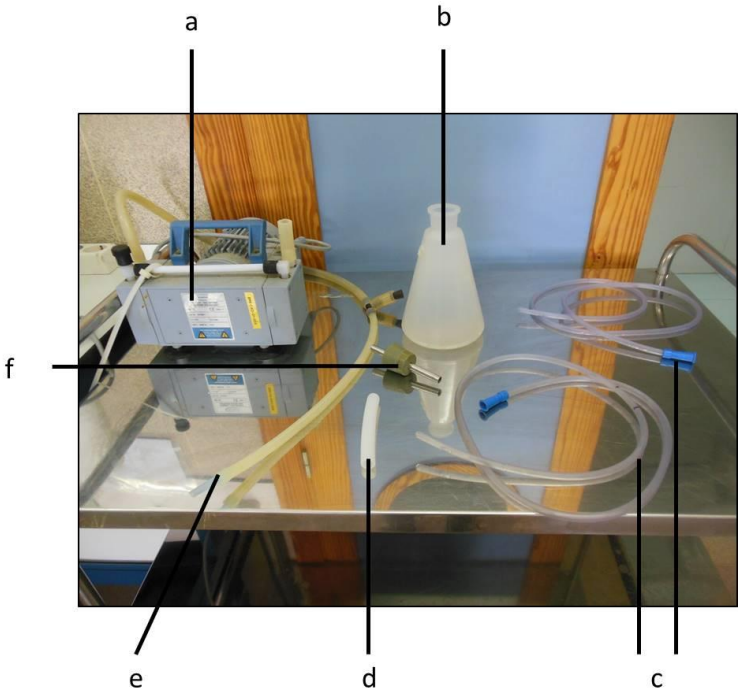

Figure 1: Materials needed to perform rumen sampling on goat kids. A: vacuum pump, b: collection erlenmeyer (hard plastic), c: flexible probing tubes, d: short, hard plastic tube, e: connection tube between pump and collection erlenmeyer, f: connection piece between probing tube and collection erlenmeyer.

- **Saliva sampling**

A little piece of a household sponge was clamped with a forceps and inserted into the mouth opening of the kid. The kid was encouraged to make chewing movements, in order for saliva production to take place. Attention was given that the kids did not bite on the forceps, so the teeth could not be damaged. After about five minutes, the forceps with the sponge was taken out en squeezed through a large pipette tip, standing in a little eppendorf tube. The saliva sample was then stored on ice.

- **Feces sampling**

Feces were sampled directly from the rectum. The kid is hold stil by one person, while the other person pulls up the tail and tries to take a fecal sample out of the rectum with one finger. Some vaseline was applied to the finger for more comfort. Feces were stored on ice.

- **Yeast culture liquid supplementation**

All the kids in the treatment group, and the mothers that stayed with half of these kids, were treated twice daily with a liquid yeast solution of *Saccharomyces cerivisiae* (Figure 2B). This was supplied by means of a so called "dosage gun" for the mothers and a smaller syringe for the kids (Figure 2A). The mothers received 50 ml of the yeast solution, the kids about 3-4 ml, and this twice daily, after feeding time. The animal is lightly restrained and the head is tilted upwards. Then the solution is slowly squirted into the mouth opening so that the animal can swallow (Figure 3). Attention is given so that the liquid does not spill out of the mouth.

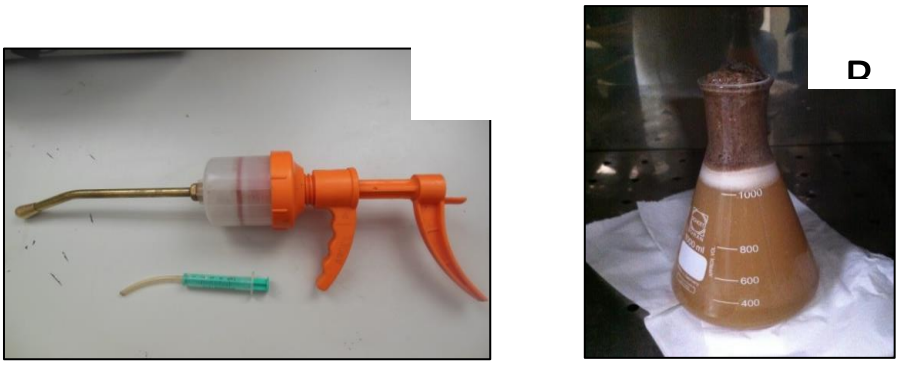

Figure 2. A: Equipment for supplementing the yeast culture solution into the mouths of goats and kids. Above the dosage gun used for the mothers, under the smaller syringe used for the kids. B: Liquid culture of the yeast *Saccharomyces cerivisiae*, in a glass erlenmeyer.

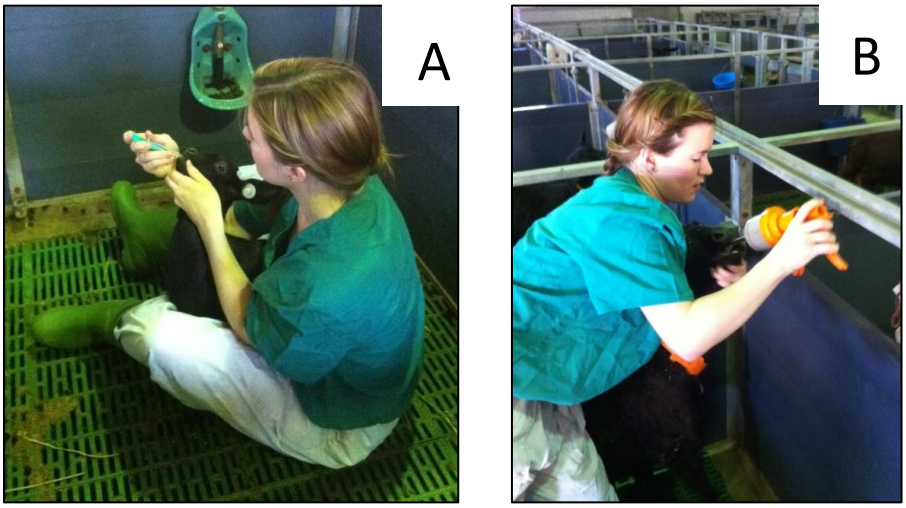

Figure 3. A: Administration of 3-5 ml of yeast culture solution to a goat kid of 10 days. B: Administration of 50 ml of the yeast culture solution to a goat mother using a dosage gun.

5. Results of activities

From this 5-day working week, I have grown more confident about taking rumen samples by oral stomach tube but also about taking saliva and fecal samples. I have had opportunities to thoroughly discuss my own experimental set up for the *in vivo* cattle and sheep trial that I will perform for my PhD study. From these discussions, I found arguments to change some key aspects of my experimental setup, by which I mean to have improved the attainability and effectiveness of my protocols.

Because I was there only one week, and at the very beginning of the trial, there are yet no results of the sample analysis.

6. Future collaboration with host institute

This mission has passed in good spirits and understanding. In the future, I will stay in contact with Dr David Yañez-Ruíz to follow up on the goat trial, but also to keep him updated about my own trials during my PhD. Because all research groups involved study the mechanisms of early life programming in the context of methane mitigation in ruminants, further collaboration would be beneficial and is likely.

7. Foreseen publications

From this STSM no publication will be made. However, I can refer to this mission in future publications, because this mission helped me determine some of the key aspects taken up in the experimental plan of my first *in vivo* trial.

8. Confirmation by host institution

For this, please see the pdf document written by the host, Dr David Yañez-Ruíz, attached to the email.