SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: FA1302
STSM title: Methane production kinetics of rumen inocula using a fully automated *in vitro* gas system.
STSM start and end date: 06/08/2017 to 21/08/2017
Grantee name: Alexis Ruiz González

PURPOSE OF THE STSM:

Objective:

(The objective of this study was to estimate the methane production kinetics in rumen inocula using an automated *in vitro* gas production system, in order to get familiar with the working mechanism that has been developed in SLU).

Summary:

Methane production during enteric fermentation in ruminant accounts for approximately 0.04–0.12 of the dietary gross energy intake and represents an energy loss to the animal (Holter and Young, 1992; De-Ramus et al., 2003; Yan et al., 2010). In this sense, it is important to develop strategies to monitor this emission. The *in vivo* methods for measuring methane production are very laborious and expensive (respiration chambers) or possibly inaccurate (tracer methods) and difficult to standardise. Therefore, *in vitro* methods would be useful for screening the effects of diets and specific additives on methane production. Two types of *in vitro* methods have been used for measurements of methane production; continuous culture (Czerkawski and Breckenridge, 1977) and batch culture experiments (Van Nevel and Demeyer, 1981).

In batch cultures, methane measurements are made with using end-point measurements, or occasionally two time points, and they do not take into account the dynamics of digestion and passage kinetics in the rumen. The automated *in vitro* gas production (GP) method is widely used to produce kinetics data of the degradation of feed or feed components (Pell and Schofield, 1993). Analysis of the kinetics of *in vitro* GP data used in the mechanistic rumen model allow us measure the methane at different time points, this approach could be used for predictions of methane production taking into account the dynamic nature of the rumen particle kinetics (Ramin and Huhtanen, 2012).

At LANUPRO, we would like to further develop *in vitro* systems to continuously monitor methane production, in order to better assess methane production kinetics, which is an essential parameter when studying the

evolution towards 'adaptation' against rumen methanogenesis. Hence, learning about the automated system at SLU using the automatic and continuous system, will facilitate the establishment of the set up at LANUPRO which relies on analogue sensors powered by an Arduino board. The present proposal was linked with METHAGENE-goals, looking for protocols to optimize *in vitro* CH_4 measurements, in order to improve the assessment of the large-scale CH_4 production by ruminants. Moreover, *in vitro* assessment is an easy and cheap technique that can be adopted to decrease the use of animals in experimental trials. Furthermore, this monitoring could be useful to record information to develop approaches for incorporating CH_4 emissions into national breeding strategies.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

From 07 to 11 of August at SLU. The main work carried out was to be familiar with the reserarchers working there and with the automated gas production system. In this sense, together with the research personal, we went through the automated gas production system in order to get familiar with the equitments and accessories that the system uses. As well, interpretation of the gas production data to monitor the kinetics of methane productions was assessed.

The second week of STSM could be split in terms of the work that was done at SLU:

a) **Data analysis of an *in vitro* experiment already available at SLU, in order to get familiar with the working mechanism and data analysis of the automated gas production system.**

Since two weeks were not enough to set up an complete *in vitro* experiment and contact the companies to check about the availability of the accessories. I was introduced to the automated gas production system to get familiar with data analysis and with the technical information about the working process, which will be very useful to set up *in vitro* experiments at Ghent University. The technical protocol to set up *in vitro* incubation with the automated gas production system requires adding substrates approximatelly 1000 mg of dried grass into serum bottles (Schott, Mainz, Germany, 250 ml) and incubating them until (in this case) 48 h. The gas production measurement in the automated system is recorded every 12 min and corrected to the normal air pressure (101.3 kPa) (Cone et al., 1996). Then the gases are released from the system by opening of the electric gas valve. The gas samples from the bottles are drawn by a gas tight syringe (Hamilton, Bonaduz, Switzerland) at different time points until 48 h of incubation. Futhermore, methane is determined by injecting 0.2 ml of gas into a star 3400 (CX series) gas chromatograph (Varian Chromatography, USA) equipped with a thermal conductivity detector (TCD).

b) **Checkup of the airtight system.**

Because the airtight system that the automated gas production has was of interest for us, we contacted the companies that had previously provided all the accessories for that system, in order to buy the same characteristics and quality with the accessories that SLU has been using. Of course, in our case the system will be different, we won´t record the gas production kinetics, rather we want to record the methane production kinetics using analoguous sensors. For this, the system has to be slightly modified to be able to

include these analogous sensors to the automated gas productions system. Some materials are already ordered e.g. bronze lids, rubber gaskets and connectors.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The main results from this STSM could be described in two parts:

Part 1: The data analysis of the gas and methane production kinetics estimated *in vitro* using a fully automated *in vitro* gas system is shown in the figures below. The main objective of data analysis was to learn the process of analysis and interpretation of the kinetics of gas and methane production in order to get familiar with processing. Results from the data analysis until 48 h of *in vitro* incubation for gas and methane production are shown in figure 1 and 2, respectively.

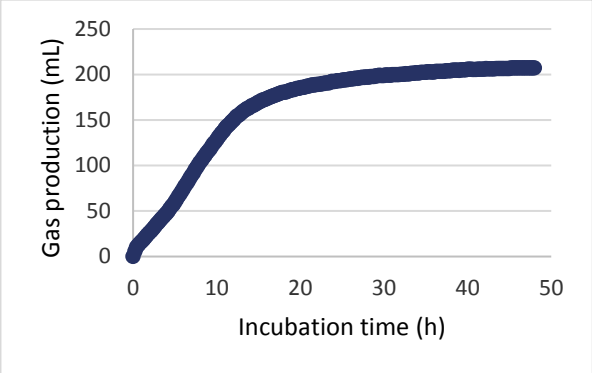

Figure 1

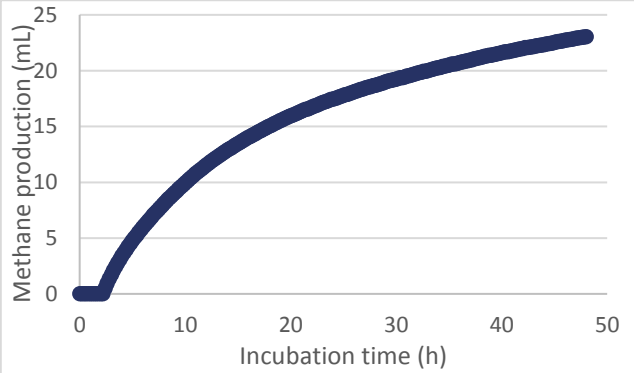

Figure 2

This exercise was very helpful to learn the process of analysis of data from the kinetics of gas and methane production.

Part 2: Checkup of the airtight system. As described in the section before - description of the work carried out, we were interested to have a look on the airtight system that SLU has developed. In this sense, we contacted the companies that sold these accessories to SLU (e.g. rubber gaskets, connectors and lids) to order these accessories to Belgium. When all the materials will be at Ghent University, we will assemble and adapt them to the methane gas sensors.

FUTURE COLLABORATIONS (if applicable)

In this sense, LANUPRO could further develop a collaborative project for a post-doctoral period of an SLU researcher at LANUPRO. If these sensors prove to be applicable to monitor the kinetics of *in vitro* methane production, the semi-continuous monitoring of methane production at SLU could be replaced by adopting the analogous methane sensors in this system.

cost
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SLU

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Institutionen för norrländsk jordbruksvetenskap

2017-08-30

TO WHOM IT MAY CONCERN

Approval letter host University – Short-Term Scientific Mission

I hereby approve the STSM report by Alexis Ruiz González, PhD student at Ghent University. Alexis Ruiz González successfully completed the STSM from 06-21/08/2017 at our department of Agricultural Research for Norther Sweden (SLU).

Sincerely yours,

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