Slovak Academy of Sciences

Institute of Animal Physiology

COST Short-Term Scientific Mission (STSM) Report

STSM Applicant:	Monika Holodová
COST Action:	FA 1302- Large-scale methane measurements on individual ruminants for genetic evaluations (METHAGENE).
Home Institution:	Slovak Academy of Sciences, Institute of Animal Physiology (IAP), Kosice, Slovakia
STSM period:	10/10/2017 to 10/11/2017
Host Institution:	Department of Animal Nutrition and Feed Management, Poznan University of Life Sciences- Prof. Adam Cieślak, Prof. Małgorzata Szumacher-Strabel

1. Background and purpose of STSM

My interest in the METHAGENE project is focusing on nutritional factors affecting methane production and methane emission in relation to animal nutrition and digestion. My PhD thesis is focused on modulation of rumen fermentation and the nutrient digestibility by phytogenic extracts and organic mineral additives to increase of animal productivity as well as to reduce the environmental pollution.

The purpose of my STSM with the working group of Prof. Adam Cieślak and Prof. Małgorzata Szumacher-Strabel at the Department of Animal Nutrition and Feed Management, University of Life Sciences in Poznan was to examine nutritional factors affecting methane production in dairy cows fed diets with different forage source. The primary objective of this internship was to examine the effect of two diets on methane production (*in vitro*) and emission (*in vivo*) in high-yielding dairy cows. We examined the effect of two diets: (i) high maize and grass silage diet (Farm 1) and (ii) high maize and alfalfa silage diet (Farm 2) on milk production and methane emission of dairy cattle using *in vitro* and *in vivo* techniques. The topic of my experiments contributes to the goal of COST Action FA1302- Methane-determining factors.

The aim of the research was monitoring of the methane production and emission from dairy cows fed diets commonly used in commercial conditions in Poland.

2. Description of the work carried out during the STSM

During the first week, we performed *in vitro* Hohenheim Gas Test experiment where the TMRs with different types of main forage (maize silage *versus* grass silage) were tested.

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Experiment *in vitro*:

Hohenheim Gas Test was used to verify the dietary forage source on *in vitro* rumen methane production. Rumen fluids were collected from three cannulated dairy cows. The diets consisted of maize silage with grass silage or maize silage with alfalfa silage as the main forage. The syringes marked according to the treatment group were incubated, and then the feeds were transferred into appropriate syringes, the syringe plungers were added and expel air was carefully pushed. The syringes were hold in an incubator at 39°C until the start of the experiment. Syringes medium was prepared 24 h before beginning the experiment. Syringes containing buffered rumen fluid were incubated for 24 h in an incubator and the gas production was recorded by changes in gas volume in the glass syringes at regular time intervals (0, 2, 4, 6, 8, 10, 12, 16, 18, 20, 24 h). After 24 h of fermentation *in vitro*, the concentration of methane and carbon dioxide in rumen fluid samples were analysed by gas chromatography in SRI PeakSimple model 310 (Alltech, PA, USA).

Photo 1. Marked syringes with the TMR samples

Photo 2. Syringes with the TMR before incubation

Photo 3. Transfer of buffered rumen fluid into syringes

Photo 4. Measurement of gas content by GC

During the next weeks of my stay, *in vivo* experiment under commercial farm conditions was performed where the same diets as in *in vitro* condition were used.

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Experiment *in vivo*:

120 milking dairy cows were divided into two dietary treatments (n=60 per treatment) according to milk yield of 36 kg per day (± 2 kg) for 2 weeks duration. Emission of CH_4 and CO_2 were determined using two non-dispersive infrared spectroscopy (NDIR) analyzers operating in the near infrared spectrum (SERVOMEX 4100, SERVOMEX Ltd, UK, detector 1210 Gfx). The unit analyzed the concentration of selected gasses in the air, sampled from the interior of the feeding bin installed in the milking robot. The collected samples were ducted to the analyzer via a polyethylene tube with an 8-mm diameter. A pump took sampling continuously with an efficiency of 15 L/min and with the sampling rate 0.6 L/min. The results of the analysis of the concentration of methane and carbon dioxide in the exhaled air was transmitted to the PC computer connected to analyzers and data were saved as text files using a widely available software.

Photos 5, 6. Infrared spectroscopy analyzers SERVOMEX 4100 on the farms

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Photo 7. Experimental milking dairy cows used in the *in vivo* experiment

Dietary treatments for all experiments were total mixed rations offered *ad libitum* and containing either maize silage with grass silage (Farm 1) or maize silage with alfalfa silage (Farm 2) as the main forage components. The chemical composition of the diets used *in vitro* as well as *in vivo* were analysed for basic components i.e. dry matter, organic matter, crude protein, ether extract, NDF, ADF (AOAC, 2007).

3. Description of the main results obtained

The preliminary data given in tables and figures presented below show that gas production and emission depends on the type of diet fed and the forage to concentrate ratio. Diet composed of maize silage with grass silage (Farm 1) containing 71% forage and 29% of concentrate produced less methane and more ammonia when compared to maize silage with alfalfa silage (Farm 2) containing 83 % forage and 17% of concentrate. There are many factors that can influence methane production. In our study, not only forage type (grass silage *vs.* alfalfa silage) but also different proportion of forage to concentrate (29% *vs.* 17% concentrate in the total diet) were used. Both factors should be taken into account explaining the reasons of methane production mitigation.

Moreover, the obtained results are in line with the theory that more concentrate diets produce less methane, however we will verify the in vitro results after finishing the calculation of all

data obtained from the farm conditions. Also in in vitro study, more analysis will be completed in rumen fluid after incubation (e.g. bacteria, protozoa and methanogens population, in vitro dry matter digestibility, volatile fatty acids etc.).

Table 1. Ingredients of TMR used in in vitro and in vivo studies

Item	Farm 1	Farm 2
	Dry matter intake (kg)	
Corn silage	8	7.4
Grass silage	2.9	-
Alfalfa silage	-	5.9
Wet distillers' grains	-	2.1
Brewery	1.8	1.3
Beet pulp	2.3	1.5
Carrot	-	0.3
Rape seed meal	-	1.8
Wheat meal	2.3	1.5
Commercial concentrate	3.4	-
Mineral supplements	0.358	0.358
Total	**21.06**	**22.16**
Forage:Concentrate	**71:29**	**83:17**

Table 2. pH, ammonia concentration and methane production after 24 h incubation of TMR from FARM 1 and FARM 2

Items	TMR - Farm1	TMR - Farm 2	SEM	P-value
pH	6.66±0.046	6.57±0.053	0.013	<0.0001
Ammonia (mmol/l)	11.81±1.49	9.45±1.05	0.319	<0.0001
Methane (mM)	10.83±0.59	12.09±0.77	0.172	<0.0001
TGP/ml(24h)	83.3±2.865	94.33±3.20	1.163	<0.0001

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Figure 1. Total gas production during 24 h of incubation

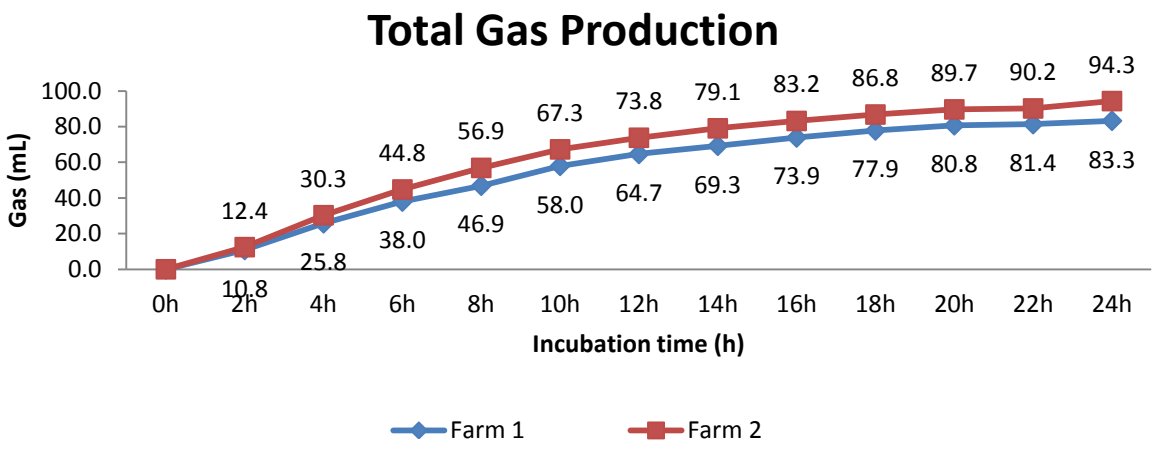

Figure 2. Example of methane (CH_4) concentrations changes during measurements in FARM1 from 18.00 of 20th of October to 00.00 21st of October

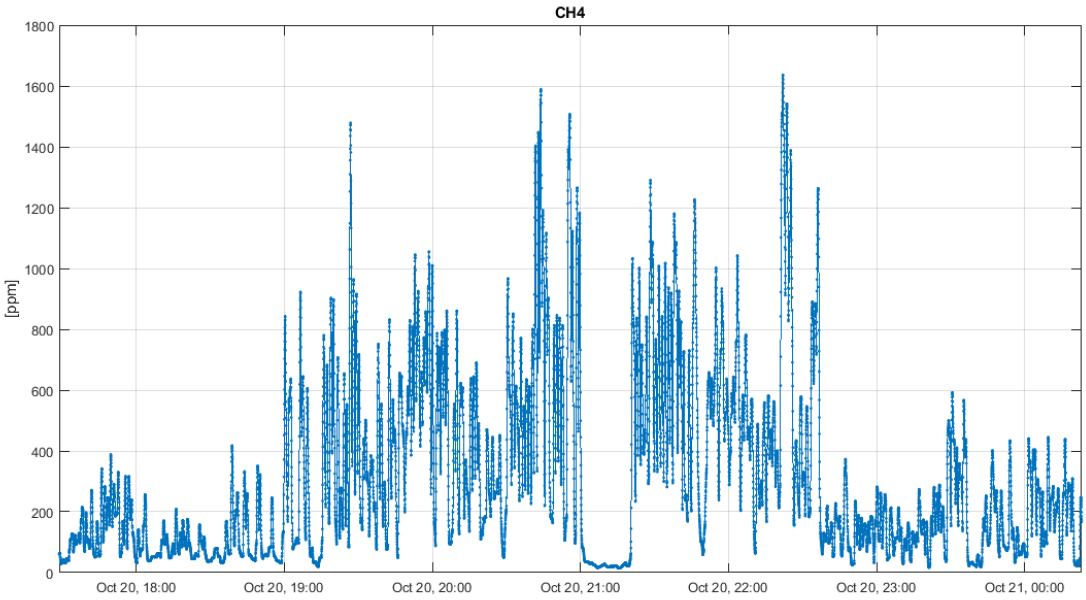

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Figure 3. Example of methane (CH_4) concentrations changes during measurements in FARM1 from 17th to 23rd of October

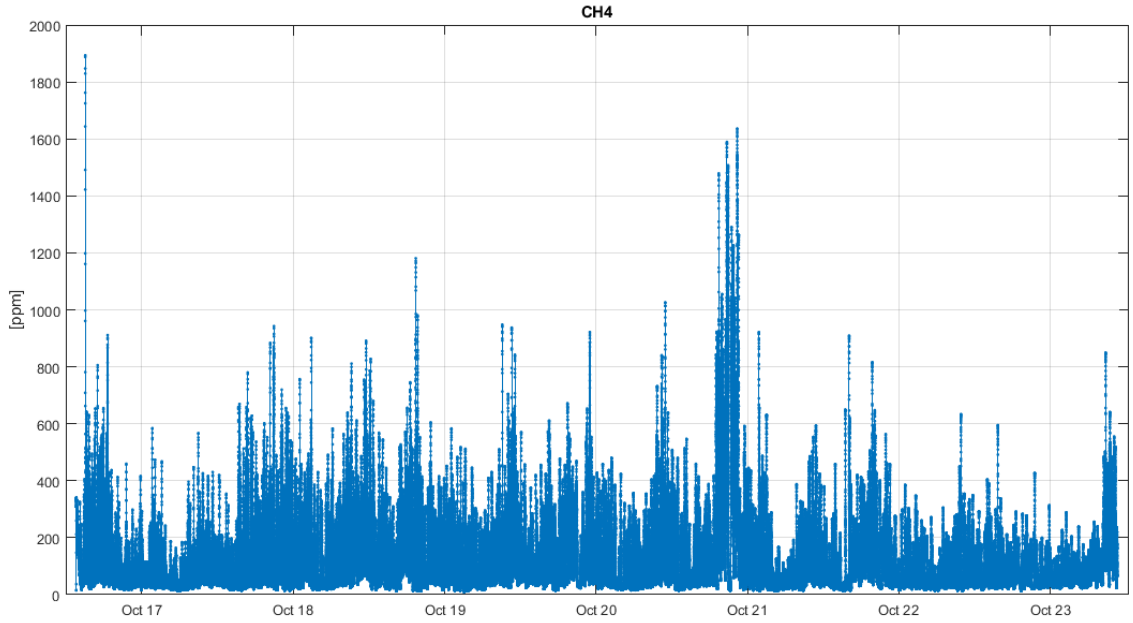

Figure 4. Example of carbon dioxide (CO_2) concentrations changes during measurements in FARM1 from 18.00 of 20th of October to 00.00 21st of October

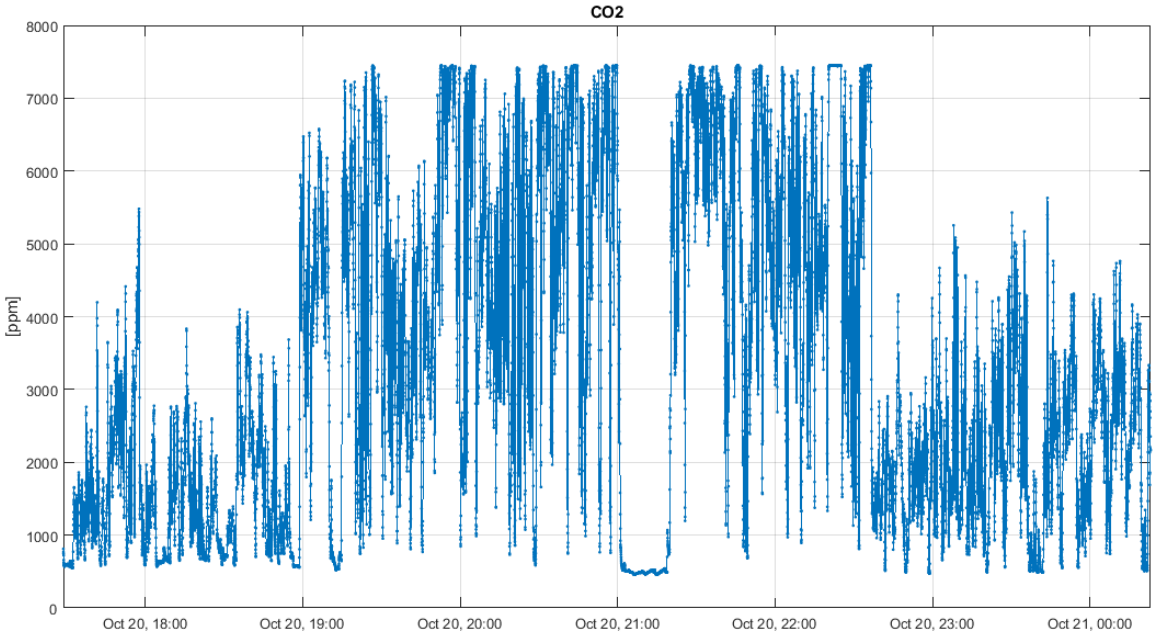

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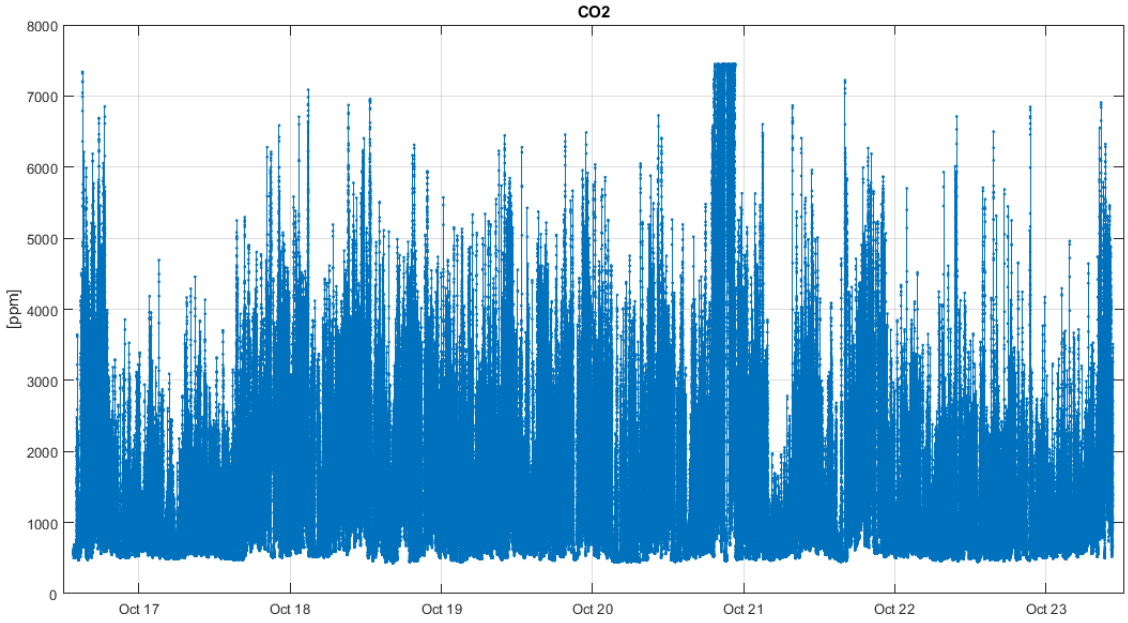
Figure 5. Example of carbon dioxide (CO_2) concentrations changes during measurements in FARM1 from 17th to 23rd of October



4. Benefits of the STSM

During the STSM at the Department of Animal Nutrition and Feed Management in Poznan I learned to perform the Hohenheim Gas Test and various experimental and analytical techniques concerning ruminal methanogenesis.

This experience provided me the opportunity to work in an international research group under supervision of Prof. Adam Cieślak and Prof. Małgorzata Szumacher-Strabel, to discuss issues and improve my knowledge in field of rumen physiology, ruminant nutrition and modulation of rumen digestion by natural additives to decrease rumen methanogenesis. I am confident that my experience at the Poznan University of Life Sciences was valuable for my PhD studies and overall general development.

In conclusion, I would like to thank all people at this Department because they were very friendly and helpful during whole my stay and I could learn a lot from them.

I would like to acknowledge the financial support by the STSM Grant from the COST Action FA1302.

5. Confirmation by the host institution of the successful execution of the STSM

Confirmation of the successful STSM execution is submitted as an e-mail attachment.

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