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Title : *In vitro* prediction of methane production by lactating dairy cows

ABSTRACT

As the world human population continues to increase, as well as affluency and urbanization, there is (and will be) a growing demand for animal derived food products. This is the case in developing countries, but also in developed countries. Ruminant animals are a major source of high quality protein (milk and meat) for humans and they have a competitive advantage compared to other animal species (e.g. pigs and poultry) that they can utilise human inedible forages for nutrition. However, ruminants are also a major contributor to the production of enteric methane (CH_4) in the world, contributing considerably to greenhouse gasses. Anaerobic microbial fermentation of feed in the rumen is accompanied by the production of CH_4, resulting in a loss of energy to the animal.

In the literature, several studies have reported CH_4 emissions from dairy cattle feeds and feed ingredients as well as additive using *in vitro* techniques. Many of these studies also investigated the possibility to reduce CH_4 emissions from rumen fermentation. However, literature reporting the relationship between *in vitro* and *in vivo* CH_4 emission are very scarce. There are no reports investigating simultaneously the CH_4 synthesis *in vitro* and *in vivo*, using the same animals and same feed ingredients and rations.

In experiments, performed at Wageningen University, the *in vivo* CH_4 emission by lactating cows on different feeding regimes was determined using climate controlled respiration chambers. Experiments were performed with grass silages differing in maturity and fertilization level, maize silages differing in stage of maturity, as well with sainfoin, a tannin-containing forage. These *in vivo* experiments are very expensive, laborious and time consuming. If *in vivo* CH_4 emission could be estimated using equations based on *in vitro* data, more rapid gains in our

knowledge and understanding of factors influencing CH_4 emission from dairy cows could be achieved. As such, performing such costly *in vivo* experiments offers a unique opportunity to determine the relationship, if any, of *in vivo* CH_4 emissions by dairy cows to *in vitro* CH_4 production using an adapted version of the gas production technique. By performing *in vivo* and *in vitro* experiments simultaneously, the rumen fluid from the cows used in the *in vivo* experiments can be used for the *in vitro* experiments. In this manner, the rumen fluid used in the *in vitro* experiments is fully adapted to the experimental rations, as is used in the *in vivo* experiments.

The aim of this thesis was to determine the relationship (if any) between *in vivo* CH_4 production obtained using climate controlled respiration chambers and *in vitro* CH_4 production using the gas production technique. In **Chapter 2**, an *in vitro* experiment is described to quantify the total rumen fermentation (gas production) and CH_4 production of grass silages, differing in maturity at harvest (early maturity, mid maturity and late maturity) and differing in nitrogen fertilisation level (65 kg of N/ha , LF; and 150 kg of N/ha HF, respectively). The *in vitro* CH_4 production was compared to the *in vivo* enteric CH_4 production. The grass silages were fed as part of a total mixed ration (TMR) to lactating cows in the *in vivo* experiment. From the *in vivo* experiment, 12 rumen fistulated cows were used as donor cows of rumen inoculum for the *in vitro* incubations. *In vitro* gas and CH_4 production was determined using rumen fluid from cows consuming each of the six different grass silages. The results indicate that the *in vitro* gas production decreased with increasing maturity of the grass at harvest. However, the stage of maturity of the grass at harvest did not affect the *in vitro* CH_4 synthesis. The *in vitro* CH_4 data correlated poorly with the *in vivo* CH_4 data. Including chemical composition and *in vitro* gas production parameters in a stepwise regression resulted also not in a correlation between the observed *in vivo* CH_4 synthesis, expressed in g/kg fat and protein corrected milk, and the predicted CH_4 production ($R^2 = 0.40$; $P = 0.36$). The results of this experiment show that *in vitro* gas and CH_4 parameters do not accurately predict *in vivo* CH_4 emissions in grass silages.

In **Chapter 3** an *in vitro* experiment is described to determine the total gas and CH_4 production of grass silages differing in maturity at harvest. The *in vitro* experiment was performed, using rumen fluid from dairy cows fed those grass silages, in early and late lactation. The *in vitro* CH_4 data, obtained with the gas production technique, were compared to the obtained *in vivo* CH_4 data using climate controlled respiration chambers. The adaptation of the rumen microbes to the grass silage based rations fed, was also investigated. Silages were made from grass harvested in 2013 at four different stages of maturity, on May 6th, May 25th, July 1st and July 8th. The grass silages were used to formulated different rations, which were fed to 24 lactating cows in early and late lactation, which resulted in a different intake (16.5 vs 15.4 kg/d

DMI) by the cows. The results show that the *in vitro* gas and CH_4 production (expressed in ml/g OM incubated), decreased with increasing maturity of the grass at harvest. The grass samples showed a higher *in vitro* gas and CH_4 production using rumen fluid from cows at a late stage of lactation than at an early stage of lactation. No correlation was observed between the *in vitro* CH_4 production (expressed in ml/g OM) and the *in vivo* obtained CH_4 production (expressed in g/kg OM intake or g/kg DM intake). Stepwise multiple regression, including the chemical composition and gas production parameters, when predicting the *in vivo* CH_4 production expressed in (g/kg OM intake) resulted in a relationship with $R^2 = 0.48$ and $P = 0.057$.

In **Chapter 4** an experiment is described to determine the *in vitro* total gas production and CH_4 production of maize silages differing in maturity, using rumen fluid from cows adapted to those maize silages. For the *in vivo* experiment, cows were fed with rations with the different maize silages. The maize silages were harvested at dry matter percentages of 25, 28, 32 and 40. Eight cows, fully adapted to their respective experimental rations served as donor cows for rumen inoculum for the *in vitro* incubations. The *in vitro* gas production was not affected by the maturity of the whole-plant maize silage, irrespective whether the maize silage itself or as part of the TMR was incubated in the adapted rumen fluid. There was no relationship between *in vitro* and *in vivo* CH_4 production from the maize silages based rations.

In **Chapter 5** *in vitro* experiments were performed to determine the total gas production and CH_4 production of samples of sainfoin silage-based and grass silage-based TMRs, using rumen fluid from cows adapted to the respective TMRs. The results indicate that *in vitro* gas production, CH_4 production and total volatile fatty acid production were not affected by the dietary treatment (sainfoin vs grass). There was no relationship between *in vivo* CH_4 production and *in vitro* CH_4 production ($R^2 = 0.02$; $P = 0.481$).

In conclusion, the lack of a relationship between *in vivo* and *in vitro* CH_4 production was observed in all experiments. Stepwise multiple regression, including the chemical composition and gas production parameters determined across feeds investigated in the Chapter 3 to 5, improved the relationship but the resulting equation did not include gas production parameters. This lack of a significant correlation with *in vitro* CH_4 production parameters could result from several factors. In *in vitro* systems, rumen fluid is commonly diluted with a buffer. Furthermore, the used *in vitro* gas production system is a closed system with no removal of fermentation end products, no supply of substrate and no simulation of rumen passage. In experiments in respiration chambers, CH_4 originates from fermentation in the rumen, but also from fermentation in the large intestine and minor amounts from the manure. The latter two are not simulated in the *in vitro* technique. Moreover, the rumen is a continuous fermentation vessel with

input (feed) and removal across the rumen wall and passage to the intestine. Nevertheless, although not found in the present work, one would expect, at least, a weak to moderately positive relationship between *in vitro* CH_4 production and *in vivo* CH_4 production. However, this could not be proven in the present work.