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Measurement and Estimation of Ruminant Methanogenesis Using the *In Vitro* Gas Production Technique

M.O. Okpara[1], C.F. Egbu[2] and U.N. Okonkwo[2]
[2]Department of Animal Nutrition and Forage Science, Michael Okpara University of Agriculture, Umudike, Nigeria
[2]Department of Agricultural Education, Alvan Ikoku Federal College of Education, Owerri, Nigeria
Corresponding author: M.O. Okpara; E-mail: morganokpara@gmail.com

Abstract
This paper is an introduction to the *in vitro* gas production technique(IVGPT) used in the measurement and estimation of ruminant methane emissions. This review compares the principles as well as the merits and inefficiencies of the IVGPT. This paper will therefore be useful for expanding the knowledge base of researchers, farm planners, and policymakers as they work to develop and maintain sustainable environmental conditions for livestock farming system.

Keywords: Methanogenesis, methane emissions, ruminants

Introduction

Over the last decade, there has been growing international interest in emissions of greenhouse gases (GHG). Greenhouse gases are atmospheric gases that absorb and re-emit long-wave radiation back to the earth surface. The main GHG's are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide. Faced with increasing evidence that the World' climate is getting warmer (IPCC, 2007), there is now an international effort to reduce anthropogenic GHG emissions to the atmosphere with the United Nations Framework Convention on Climate Change (UNFCCC) and the Kyoto Protocol (Clark *et al.,* 2011) being the most important organizations.Ruminants have the unique advantage of converting otherwise indigestible cellulose rich plant materials into meat, milk, wool and other products, whilst not competing with humans directly for food (Buddle *et al.,* 2011).

However, farming ruminant livestock is associated with environmental impact, and CH_4 emissions from enteric fermentation are major contributor to GHG emission. The principal source of CH_4 from ruminants is enteric fermentation arising from digestive processes in the rumen, and to a lesser extent, the large intestine (Clark *et al.,* 2005; Waghorn *et al.,* 2006).The IVGPT has been used to stimulate ruminal fermentation of feeds and feedstuffs (Rymer *et al.,* 2005) for decades. With the increasing interest in GHG emissions from agriculture in recent years, the traditional IVGPT have been modified to include measurement of CH_4 production.

This review summarizes the IVGPT, advantages and disadvantages of this technique for easy understanding.

Principle of IVGPT

The gas measuring technique has been widely used for evaluation of the nutritive value of feeds. More recently, the increased interest in the efficient utilization of roughage diets has led to an increase in the use of this technique due to the advantage in studying fermentation kinetics. Gas measurement provides a useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs (France *et al.,* 2000).The basic principle of IVGPT is to ferment feed under controlled laboratory conditions employing rumen microbes. Feedstuffs, e.g., subjected to different treatments, are incubated at 39^{0}C with a mixture of rumen fluid, buffer and minerals for a certain time period, typically 24, 48, 72, 96 or 144h. The amount of total gas produced during incubation is measured and its composition analyzed, to obtain data on the *in vitro* degradation of the feedstuffs, making it possible to determine whether a reduction in CH_4 production is at the cost of total feed degradation. The output of IVGPT experiments is usually reported as amount of CH_4 per gram dry matter (DM), per gram degraded DM (dDM) or per gram degraded NDF (dNDF).

Collection of rumen fluid

The method requires access to fresh rumen fluid, which is typically obtained from fistulated cows or other ruminants. Alternative methods of collecting rumen fluid are by oesophageal tubing on intact animals or from slaughtered animals. The use of feces or cultures as alternative inoculants has been compared to fresh rumen fluid only for total gas production/feed degradation (Mould *et al.,* 2005). Such alternative sources of inoculums are not expected to be applicable for estimation of CH_4 production because of the complexity of the microbial ecosystem.

Advantages and inefficiencies of IVGPT

The typical time frame for conducting an *in vitro* experiment is 1–4 weeks, which makes it possible to screen many different feedstuffs and potential additives relatively fast and cheaply. Compared to *in vivo* experiments, it is also much easier to control fermentation conditions like pH. The cow-to-cow variation observed *in vivo* can be avoided by using the same ruminal

inoculum for all treatments which are to be compared *in vitro*. Preferably the inoculum is produced by mixing rumen fluid from several donor animals to include as many different rumen microbes as possible.

Also,depending on the system and other laboratory constraints it is possible to conduct up to several hundred parallel incubations at a time, which allows for sufficient amounts of repetitionsin experiments to support statistically significant differences between treatments.

A clear disadvantage of IVGPT is that it only simulates the ruminal fermentation of feed, not emissions and digestibility by the entire animal. Furthermore, under normal conditions it does not include long-term adaptation of the ruminal microorganisms to the tested feedstuffs. It is common to use rumen fluid from animals on a standard feed ration. During live animal experiments it is common practice to have adaptation periods to new feeds of at least 14 days. The animals' output is not considered stable before that. For the CH_4producing population of ruminal microbes there are indications that the adaptation period after switching to a new feed is more than 30 days (Williams *et al.,* 2009).

Conclusion

The IVGPT is a good method of measuring and estimating CH_4 emissions from ruminants. It is however, not flawless and therefore requires careful consideration before application. In this context, a thorough knowledge of the advantages and disadvantages of IVGPT is extremely important, both when planning experiments and when interpreting own results and the published findings of other researchers.

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