

In vitro technique

Sangkhom INTHAPANYA and TR Preston

inthapanyasangkhom@gmail.com

preston@lrrd.org

Souphanouvong University, Lao PDR

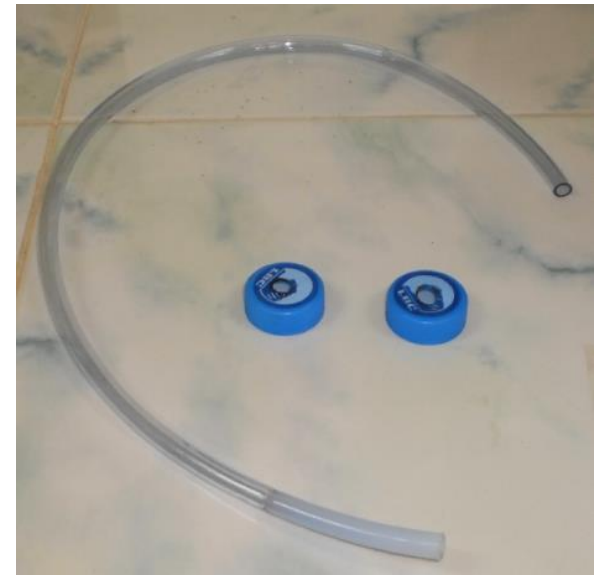
2014

Introduction

- ***In vitro* fermentation procedures to evaluate the nutritive value of feeds promoted by Tilly and Terry (1963)**
- **Ørskov and Hovell (1980) and Menke and Steingass (1988).**
- **Recently by Khan and Chaudhry (2009) to evaluate the effect of spices as potential modifiers of methane production in diets for ruminants.**
- **Developed and use a simple *in vitro* method to screen the potential methane production from a diet based on cassava root as the energy source supplemented with protein from mimosa or cassava leaves, using calcium nitrate and urea as sources of non-protein nitrogen (Sangkhom et al 2011).**

The in vitro system (1/2)

- **The simple way to determine the gas production by using the recycled water bottle (1500 ml)**
- **Making a hole in the lid of every bottle and connecting one bottle to the other one by a plastic tube through the hole in order to transfer gas during incubation.**



The in vitro system (2/2)

- **The receiving bottle suspended in water is marked by 50ml intervals to indicate the amount of gas production by displacement.**
- **After adding buffer solution and rumen fluid to samples of the substrate into each bottle and filling each bottle with carbon dioxide the clay is used to cover the lid in every bottle for preventing the gas leak.**





2



3



1



4



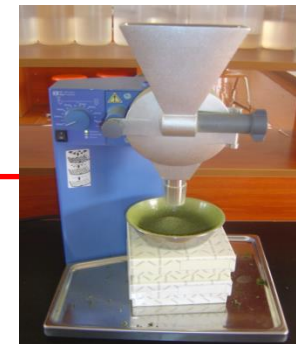
6



5

Preparation of diets

- **Foliage (root and leaf) 0.5 - 1 cm of length and dried in an oven 105°C and milled through a 1 mm screen.**



Preparation of buffer solution

- 0.96 liters (
- 100 ml) of buffer solution (water bottle 1500ml) (100ml)

Ingredients of the buffer solution

| Ingredients | CaCl ₂ | NaHPO ₄ ·12H ₂ O | NaCl | KCl | MgSO ₄ ·7H ₂ O | NaHCO ₃ | Cysteine |
|-------------|-------------------|--|------|------|--------------------------------------|--------------------|----------|
| (g/liter) | 0.04 | 9.30 | 0.47 | 0.57 | 0.12 | 9.80 | 0.25 |

Source: Tilly and Terry 1963



Preparation of rumen fluid

- **240 (150)ml of rumen fluid (from slaughter house).**
- **The bottle then incubated at 38 °C in a water bath.**





Taking the rumen fluid by stomach tube

Procedure

- **The samples of the mixtures (12 g DM) in Water bottle of 1500 ml.**
- **Added 0.96 liters) (100 ml) of buffer solution and 240 ml (150ml) ml of rumen fluid prior to filling each bottle with CO₂ and then each bottle covered by clay for preventing air leak.**
- **The bottle incubated aml)t 38 °C in a water bath. During the incubation, each bottle connected to a bottle suspended in water to collect the gas by displacement.**



Clay

Measurements



1. Gas production after fermentation



Scissors



**2. %, CH₄
measured by
Crowcon infra-
red analyser**



3. Each water bottle filtered by white cloth (particle sizes to at least 0.1mm) and dried (100c for 24hours) in oven for finding DM substrate fermented

Procedure

- **Set up the in vitro system**
- **Prepare the substrates**
- **Prepare the buffer solution & rumen fluid**
- **Materials for measure the methane in the gas**
- **Measure the DM residue after fermentation**

El sustrato

Composición (base seca)

- Raiz de yuca 73%
- Urea 2%
- Hojas arboreas 25%
- Minerales 1%

El Ensayo

Medir la producción de metano y la digestibilidad en un sistema alimenticio basado en raíz de yuca-urea y cuatro fuentes de proteína arbórea:

- Moringa
- Morera
- Yuca
- Tithonia

Diseño experimental

- 4 tratamientos con 4 repeticiones
- Mediciones de producción de gas y su porcentaje de metano cada 6 horas hasta 24 horas en total
- Al fin de 24 horas se determina la materia seca del residuo.

Fuentes de proteína

- Hojas de yuca amarga
- Hojas de Moringa
- Hojas de Morera
- Hojas de *Tithonia diversifolia*

**Thank you for your
attention**