13.1 *In vitro* gas production technique

A system for feed evaluation based on *in vitro* gas production was developed in Germany by Menke *et al.* (1979) and Menke and Steingass (1988). This system is basically a Tilley and Terry (1963) system but in this method, gas production rather than dry matter loss measured.

The amount of gas (CO_2 and CH_4) released when feeds are incubated *in vitro* with rumen liquor is closely related to digestibility and to the energetic feed value of diets for ruminants. The feeds of different digestibility produce different volume of gases with in a stipulated time. A sample is introduced into a calibrated glass syringe with buffer rumen liquor medium and incubated at 39ºC. At the end of incubation the gas produced is recorded from glass syringe. Unlike Tilley and Terry where disappearance of substrate is measured in this method fermentation production i.e. gas is measured.

Equipment

- Incubator
- Glass syringes 100 ml (graduated 1/1, with capillary attachment)
- Silicone tube (50 mm per glass syringe)
- Analytical balance
- Suction pump
- Bottle with stopper
- CO_2 cylinder
- Filling equipment for rumen fluid

Fig. 13.1 Incubation of samples by in vitro gas production technique

Reagents

1. Main element solution

Na_2HPO_4	-	2.70 g
KH_2PO_4	-	6.2 g
$MgSO_4.7H_2O$	-	0.6 g

Make up to 1 L with distilled water

2. Trace element solution

$CaCl_2.2H_2O$	-	13.2 g
$MnCl_2.4H_2O$	-	10.0 g
$CoCl_2.6H_2O$	-	1.0 g
$FeCl_2.6H_2O$	-	0.8 g

Make up 100 ml with distilled water

3. Buffer solution

$NaHCO_3$	-	35 g
NH_4CO_3	-	4 g

Make up 1 L with distilled water

4. Resazurine solution

Resazurine	-	100 mg
Distilled water	-	100 ml

5. Reduction solution

1 N NaOH	-	2 ml
$Na_2S.7H_2O$	-	0.285 g
Distilled water	-	47.5 ml

It should be prepared fresh at the time of use.

Procedure

Day 1

- Prepare all the solutions except reducing solution
- Feed sample should be prepared by milling using 1 mm screen and weigh 200 mg dry feed.
- Prepare syringes in the following way:

  Weigh 200 mg sample in each numbered syringes

  After weighing, grease the plungers with Vaseline and place in incubator at 39ºC

  Keep three syringes without feed for incubation as blank.

Day 2

- Prepare reduction solution as mentioned in reagents.
- Prepare medium by mixing the ingredients sequentially as given below:

Water	-	400 ml
Micro mineral solution	-	1.0 ml
Buffer solution	-	200 ml
Macro mineral solution	-	200 ml
Resazurine solution	-	1.0 ml
Reduction solution	-	2.40 ml

a. Maintain the medium at 39ºC then add reducing solution

b. Put magnet in the flask and gently bubble CO_2 through the solution until the blue color turns to pink and then clear.

c. After bubbling raise the CO_2 tube above the level of the contents of the flask and stream CO_2 throughout the dispensing procedure.

- Collect rumen fluid from animal.
- Strain through cheese cloth/gauze in a warm (39ºC) beaker.
- Prepare the rumen fluid and medium mixture:

a) Add rumen fluid to the medium when it becomes colorless at the ratio of 1:2 (v/v).

b) Bubble CO_2 for 15 min and then with raised tube during filling.

- Transfer 30 ml rumen fluid-medium mixture with semiautomatic pipette in preheated (39ºC) syringe containing 200 mg feed.
- Fix one and end of the rubber tube properly to the attachment of the syringe and another end to the pipette to join the syringe and pipette.
- Remove the air bubbles by gentle shaking and moving the piston upward.
- Shut the clamp on the tube and record the volume of the mixture in the syringe.
- Incubate the syringe at 39ºC (Fig. 13.1).
- Record the gas volume after 24 h.
- Mix gently each syringe 2-3 times during first day, as well as at the time of reading.
- Reading time can be selected to suit the type of substrate tested. For forages 3, 6, 12, 24, 48 and 96 h are suitable but for concentrate more readings in first 24 h are required.
- The sample should be done in triplicate.
- Three syringes containing only rumen fluid buffer solution with no feed sample are processed as blank and three syringes with standard hay sample are also incubated to monitor the activity of rumen fluid.

Calculation

$$\text{Gas production (ml/200 mg DM)} = \frac{(V - V_o - G_o)}{\text{Feed (mg)}} \times 200$$

V = Reading after 24 h of incubation

V_o = Reading just before incubation

G_o = Gas produced in blank syringes

Note: The prediction equations have been developed by which the organic matter (OM) digestibility and metabolisable energy (ME) can be estimated.

Digestible organic matter

DOM (%) = 14.88 + 0.889 * gas (ml/200 mg DM) + 0.45 * CP + 0.65 * Ash.

Metabolisable energy content of feed

For concentrates

ME (KJ/kg DM) = 1.06 + 0.157* gas (ml/200 mg DM) + 0.0084 *CP + 0.022 *EE – 0.0081*Ash

For roughages

ME (KJ) = 2.20 + 0.136 * gas (ml/200 mg DM) + 0.0057 *CP + 0.0029 *EE^2

A common equation useful both for roughage and concentrate

ME (KJ) = 1.24 + 0.146* gas (ml/200 mg DM) + 0.007 *CP + 0.0224 *EE

* CP (crude protein), EE (ether extract) and Ash in g/kg DM

Precauti
on

Sample of rumen liquor should be drawn after 2-3 h of feeding.

Anaerobic condition must be maintained throughout the

experiment.

References: Menke, K.H. and Steingass, H. (1988). Estimation of the energetic feed value obtained by chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev., 28:7.; Menke, K.H., Raab. L., Salewski, A. Steingass, H., Fritz. D and Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedstuff from the gas production when they are incubated with rumen liquor.