

***In vitro* gas production technique**

Ref: Menke; K.H. & Steingass, H. 1988. ESTIMATION OF THE ENERGETIC FEED VALUE OBTAINED FROM CHEMICAL ANALYSIS AND *IN VITRO* GAS PRODUCTION USING RUMEN FLUID. Animal Research and Development. Vol. 28

Preparation of syringes

All substrates should be milled using a 1mm screen. Weigh 200mg substrate into each (numbered) syringe and record actual weight. Include a blank (i.e. rumen fluid/artificial saliva mixture on its own) at the beginning, in the middle of the set, and at the end. Control sample, should be added in each run to correct for possible variations between runs. Samples should be done in duplicate or triplicate. After weighing is completed, grease the plungers with vaseline, and place in incubator at 39°C. This is normally done the day before the run.

Preparation of Artificial Saliva

Add distilled water, buffer solution, macromineral solution, micromineral solution and resazurin solution into round flat-bottomed flask. Warm to 39°C then add reducing solution (see table below). Place water bath set at 39°C on magnetic stirrer, put magnet into flask and gently bubble CO₂ into the solution until the blue color turns to pink then clear – this means the artificial saliva is now reduced. Raise the CO₂ tube so that it will be above the level of the artificial saliva/rumen fluid mixture, but providing a stream of CO₂ and an O₂-free atmosphere, buffer should be pH 7.0-7.3.

Collect rumen fluid from the animals (normally 2 or 3), strain rumen liquid through three layers of gauze; the final ratio of artificial saliva:rumen fluid should be 2:1. Pour the strained rumen liquid into the artificial saliva. Make sure the magnet is stirring properly during the whole process of dispensing the rumen fluid/artificial saliva into the syringes. Add 30ml solution to each syringe using a dispenser. Fill the syringe, then open the clip and gently push the syringe's plunger so that all the air is removed. Record the volume and place in water bath.

Readings can be taken to suit the type of substrate in the syringes. For forages 3, 6, 12, 24, 48, 72 and 96hr are suitable but for concentrate substrates it may be necessary to take more readings in the first 24hrs. It is advisable to gently mix each syringe 2-3 times during the first day as well as each time a reading is taken.

Description of Solutions Required for the Gas Technique

Macromineral Solution:

Na₂HPO₄ 5.7g
 KH₂PO₄ 6.2g
 MgSO₄ 0.6g
 -make up to 1 L with distilled water

Micromineral Solution:

CaCl₂·2H₂O 13.2g
 MnCl₂·4H₂O 10.0g
 CoCl₂·6H₂O 1.0g
 FeCl₂·6H₂O 0.8g
 -make up to 1 L with distilled water

Artificial Saliva:

NaHCO₃ 35g
 (NH₄) HCO₃ 4g
 -make up to 1 L with distilled water

Resazurin aqueous

(100mg/100ml)

Preparation of Artificial Saliva

Artificial Saliva - final volume	Volume (ml)			
	500	1000	1500	2000
Distilled water	237.5	475.0	712.5	950.0
Macromineral solution	120.0	240.0	360.0	480.0
Buffer solution	120.0	240.0	360.0	480.0
Micromineral solution	0.06	0.12	0.18	0.24
Resazurin	0.61	1.22	1.83	2.44
Reducing Solution				
Distilled water	23.8	47.5	71.3	95.0
1M NaOH	1.0	2.0	3.0	4.0
Na ₂ S ₉ ·H ₂ O (mg)	168	336	504	672