PRELIMINARY RESULTS ON *IN VITRO* GAS PRODUCTION IMPACTED BY DIFFERENT FAECAL SOURCES OF ANIMAL SPECIES USED AS INOCULA

Nguyen Van Thu

Department of Animal Sciences, College of Agriculture, Can Tho University, Vietnam

Corresponding author: Nguyen Van Thu; Email: nvthu@ctu.edu.vn

ABSTRACT

The objective of this study was to evaluate effect of faeces sources used as inocula on *in vitro* gas production for future applied studies. The experiment was conducted at Department of Animal Sciences, College of Agriculture of Can Tho University. It was a factorial design (5*4) with 4 replications. Factor 1 was five sources of faeces from buffalo (BF), cattle (CF), pig (PF), goat (GF) and rabbit (RF). The factor 2 was 4 feeds including Para grass (PG), *sesbania grandiflora* (SG), rice straw (RS) and pineapple peel (PP). The measurements of gas production were at 3, 6, 9, 12, 24, 48, 72 and 96 h. The results showed that gas production of five source of faeces were significantly different ($P<0.05$) among them. At 12, 24, 48 and 96 h gas production of GF was the highest values and significantly higher ($P<0.05$) compared to BF and RF. The *in vitro* gas production of PP was the highest values and significantly different ($P<0.05$) to PG, SG and RS at 24, 48 and 96 h of incubation. The conclusion was that all faecal sources of the study could be used as inocula for in *in vitro* gas production and goat faeces was the most potential for applications.

Keywords: *faeces, feed, gas production, microorganism, in vitro*

INTRODUCTION

In ruminant production, the *in vitro* gas production (IVGP) technique is a very important method to determine the gas production, digestibility and metabolism energy due to the quick results, more feeds evaluation and the cheap costs. Its principle is based on the rate of production of gases (carbon dioxide, methane, hydrogen); volatile fatty acids (acetic acid, propionic acid, butyric acid...) when fermented with microbial source (inoculum) from rumen fluid, help determine digestibility and metabolism energy (ME) value of feed. However, difficulty of the traditional method of *in vitro* gas production was the using of rumen fluid from fistulated ruminants with the costs of taking care and other costs for maintenance and labors. In the other hand, it is not the animal welfare. Nguyen Van Thu and Udén (2003) and Posada et al. (2012) suggested that using faecal microorganism sources to replace microorganism one from rumen fluid for *in vitro* gas studies was highly potential. Pandian et al. (2016) also showed that faecal inoculum could be used as an alternative to the rumen one for IVGP technique. However, the substitution of faecal microorganisms for rumen fluid in *in vitro* experiment has not been closely investigated, so it should be considered with some cautions. Especially, studies of influence of microbial sources from faeces of different animal species for *in vitro* gas evaluation have been still limited. Therefore the objective of the present study was to evaluate the ability of different faecal sources of microorganisms to produce *in vitro* gases for some recommendations of applied studies.

MATERIALS AND METHOD

Location and time

The experiment was conducted at the Laboratory E205, Department of Animal Sciences, College of Agriculture and Applied Biology, Can Tho University, from March to June, 2018.

Experimental design

The experiment was a factorial design (5*4) with 4 replications. Factor 1 was five sources of faeces from buffalo (BF), cattle (CF), pig (PF), goat (GF) and rabbit (RF). Factor 2 was four feed sources from Para grass (PG), *sesbania gradiflora* (SG), rice straw (RS) and pineapple peel (PP).

Materials and *in vitro* technique

Feed samples were collected in the Can Tho University area and surrounding areas. When sampling both the old and the young, the same sample should be collected at different locations. These feed would be cut short from 2 -3 cm to dry at 55°C for 48 hours and then ground through 1mm sieve.

In vitro gas production was done following the procedure described by Mauricio et al. (2001). Faecal samples are collected and processed as follows: 2 buffalo and 2 cattle were fed natural grasses and then faecal samples were taken directly from the rectum; while in pigs, goats, and rabbits faeces were immediately collected when the animals discharged. The collected faeces were put in a bottle to keep warm and to ensure the anaerobic conditions for microorganisms survive. Feed rations should be recorded at each experimental replication.

The collected faeces was weighed 100 g into 1 liter of mixed medium, then filtered thoroughly. The processing medium is kept in anaerobic conditions at a temperature of 39°C in an incubator. Weigh 200 mg (dry matter) of the sample into graduated syringes (3 tubes each). A sample tube is added to 30 ml of the treated medium and then incubated immediately at 39°C following the method described by Nguyen Van Thu and Udén (2003).

Measurements taken

The amount of gas produced was determined by reading directly on the syringes. The time series measurement of gas volumes were recorded from 3, 6, 12, 24, 48, 72 and 96.

Feed samples as the substrates and faeces were analysed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash according to the standard methods of AOAC (1990), while neutral detergent fiber (NDF) was analyzed following procedures suggested by Van Soest et al. (1991).

Data analysis

The data were analyzed by analysis of variance of a factorial design (5*4) with 4 replications using General linear model (GLM) of the two-way model in Minitab 16.1.0.0 software (Minitab 2010). To compare difference between mean values of treatments with the Tukey's test was used (Minitab 2010).

RESULT AND DISCUSSION

Chemical composition of feed and faeces

Table 1. Chemical composition (%) of feeds used in the experiment

Feed	DM	OM	CP	EE	NFE	CF	NDF	Ash
Para grass (PG)	19.6	89.4	10.7	4.30	45.8	28.6	66.9	10.6
Sesbania grandiflora (SG)	24.5	91.0	20.0	8.50	50.0	12.5	25.1	9.01
Rice straw (RS)	64.9	85.2	3.30	3.10	41.0	36.8	67.2	15.8
Pineapple peel (PP)	9.40	94.1	4.00	6.90	72.1	11.1	39.4	5.90

Note: DM: dry matter, OM: organic matter, CP: crude protein, EE: Ether extract, CF: crude fiber, NFE: nitrogen free extract, NDF: neutral detergent fiber.

Table 1 shows that *Sesbania grandiflora* (SG) had the highest crude protein (CP) and ether extract (EE) content (20.0 and 8.50%, respectively), the lowest values were Para grass (10.7 and 4.30%, respectively), pineapple peel (4.0 and 6.90%, respectively) and rice straw (3.30 and 3.10%, respectively). Reddy and Elanchezhian (2008) showed *Sesbania grand flora* had the CP and EE content were 24.9 and 4.68%, respectively. Crude fiber (CF) and neutral detergent fiber (NDF) content of rice straw (33.8 and 67.2%, respectively) was higher than those of Para grass (28.6 and 48.2%, respectively), SG (12.5 and 25.1%, respectively) and PP (11.1 and 39.4%, respectively). In general, pineapple peel is more digestible than straw due to its low fiber content and high non-fiber carbohydrate content (72.2 % NFE). While *Sesbania grandiflora* and Para grass were 50.0 and 45.8% NFE, respectively and lowest value for rice straw (45.0%). Heuzé et al. (2015) indicated that the NFE content of pineapple was 68.4%.

Table 2. Chemical composition of faecal sources of different animal species

Faeces sources	DM	OM	CP	CF	Ash
Buffalo	10.5	73.1	8.80	30.1	26.9
Cattle	16.7	79.6	8.66	26.1	20.4
Pig	25.7	75.7	15.2	27.8	24.3
Goat	31.2	87.3	13.7	31.5	12.7
Rabbit	31.4	89.0	9.77	38.6	11.0

The DM content of rabbit faeces (31.4%) and goat faeces (31.2%) were similar, then the gradual reduction of values for pig, cattle and buffalo (Table 2). Similarly, the CF content of rabbit faeces (38.6%) was also highest value, while the CF contents were lower for goat (31.5) and buffalo (30.1%). This result was lower than that of the report of Peiretti et al. (2014) on faeces of 148 rabbits being 32.4%. However, pig faeces had the highest CP content (15,2%), followed by faeces of goat and rabbit (13.7 and 9.77%, respectively); buffalo and cattle faeces had the lowest CP content (8.80 and 8.66%, respectively). Yen et al. (2017) on biogas production from vegetable wastes combined with manure from pigs or buffaloes had the CP content of pig and buffalo faeces were calculated at 12.0 and 6.5%, respectively. Through the analysis of composition of the same faeces source at different collection times, it was found that the diet of the feed has affect protein content of faeces, and when feeding animals with high-protein diets, then protein content of faeces was also higher than that of low-protein diets.

Gas production with different faeces sources

Table 3. Total gas production (ml/gOM) of treatments over different incubation times

		Incubation times			
		12h	***24h***	***48h***	***96h***
Faecal sources (FS)	Goat (GF)	43.3^{a}	86.9^{a}	100^{a}	110^{a}
	Cattle (CF)	29.0^{ab}	67.8^{ab}	79.8^{ab}	88.7^{ab}
	Pig (PF)	38.9^{ab}	71.4^{ab}	79.2^{ab}	83.6^{ab}
	Rabbit (RF)	27.9^{b}	55.7^{b}	69.8^{b}	77.1^{b}
	Buffalo (BF)	17.6^{b}	49.9^{b}	66.4^{b}	76.9^{b}
Feeds (F)	Pineapple peel (PP)	90.0^{A}	159^{A}	175^{A}	177^{A}
	Para grass (PG)	12.0^{BC}	43.1^{B}	63.5^{B}	73.5^{B}
	Sesbania grandiflora (SG)	20.5^{B}	53.1^{B}	59.0^{B}	62.7^{B}
	Rice straw (RS)	2.95^{C}	9.65^{C}	19.0^{C}	35.4^{C}
P	Faecal source (FS)	0.001	0.001	0.002	0.008
	Feed (F)	0.001	0.001	0.001	0.001
	FS * F	0.219	0.405	0.470	0.749

Note: $^{a, b, c}$ Means with different letters within the same column on factor faeces were significantly different at the 5% level. $^{A, B, C}$ Means with different letters within the same column on factor feed were significantly different at the 5% level.

In general gas production was gradually increased from 12 to 96 h for both faecal sources (FS) and feeds (F) in Table 3. Relating to the impact of faecal sources at 12, 24, 48 and 96 h, the gas production of GF source (43.3, 86.9, 100 and 110, respectively) was the highest values and significantly different (P<0.05) to RF treatments (27.9, 55.7, 69.8 and 77.1) and BF (17.6, 49.9, 66.4 and 76.9, respectively). However, this was not significantly different (P>0.05) to PF (38.9, 71.4, 79.2 and 83.6) and CF (29.0, 67.8, 79.8 and 88,7, respectively). Gas production of CF and PF was not statistically significant difference (P>0.05). According to the study of Pandian et al. (2016) on gas production from faecal inoculum cattle faeces with the substrate being sorghum, which was higher than those from rumen inoculum about 11.6%.

With different feeds, gas production from 12 to 96 h was significantly different among them (P<0.05). The gas production of PP was the highest value and significantly higher than that of SG, PG and RS. The gas production of PG was not significantly different (P>0.05) to that of RS and SG, while this was significantly higher (P<0.05) for SG (20.5 ml/gOM) than RS (2.95 ml/gOM). The result of gas from PP produced (90.0 to 177 ml/OM), while Pornpan et al. (2016) reported that fermenting sugar palm peel with pineapple peel in a 2:1 ratio at day 21 resulted in that gas volume from rumen fluid of cattle at 12, 24, 48 and 72 hours was 96.7, 167, 220 and 240 ml/gOM. The higher gas production could be caused by the higher NFE content of feeds (Nguyen Binh Truong and Nguyen Van Thu, 2020). The statistical analysis of this factorial design experiment also indicated that interaction of production between faecal sources and feeds for gas production was not significant different (P>0.05) during the incubation times (Table 3).

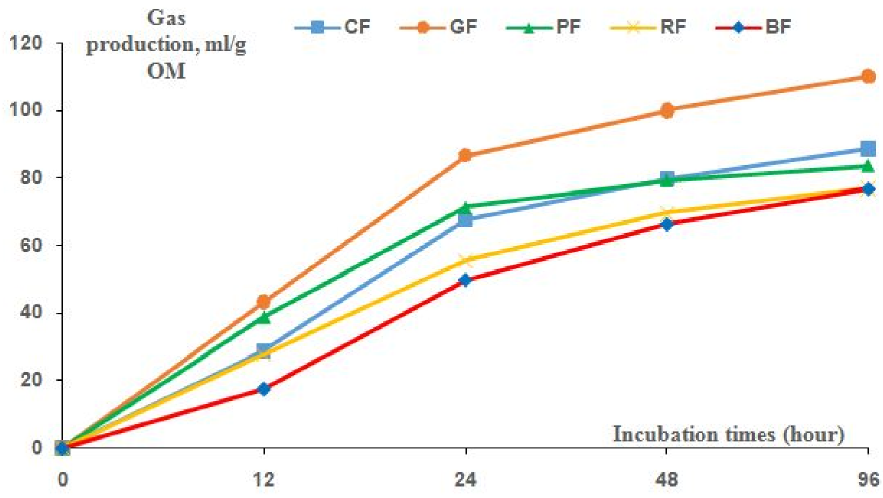

Fig 1. *In vitro* gas production (ml/gOM) from feces sources over incubation times

The *in vitro* gas production from faecal sources was presented in Fig. 1. In general, the accumulated gas increased with augmenting incubation time at 96 h. It also showed that the accumulated gas production was sharply increased in different faecal sources from 0 to 24 hours, while it was slightly increased from 24 to 96 hours (Fig. 1). Goat faeces had higher DM and CP content than cattle, buffalo and rabbit faeces. It was also indicated that the source of microorganisms from goat faeces produced the most high gas production compared to the others. Although, CP content of pig faeces was higher than those of goat faeces, pig are monogastric mammals that feed mainly not fibers and the role of microorganisms in their digestion is negligible. Degen et al. (2010) studied on *in vitro* gas production with different inocula found that goat rumen fluid produced the most high gas compared to cattle and sheep ones.

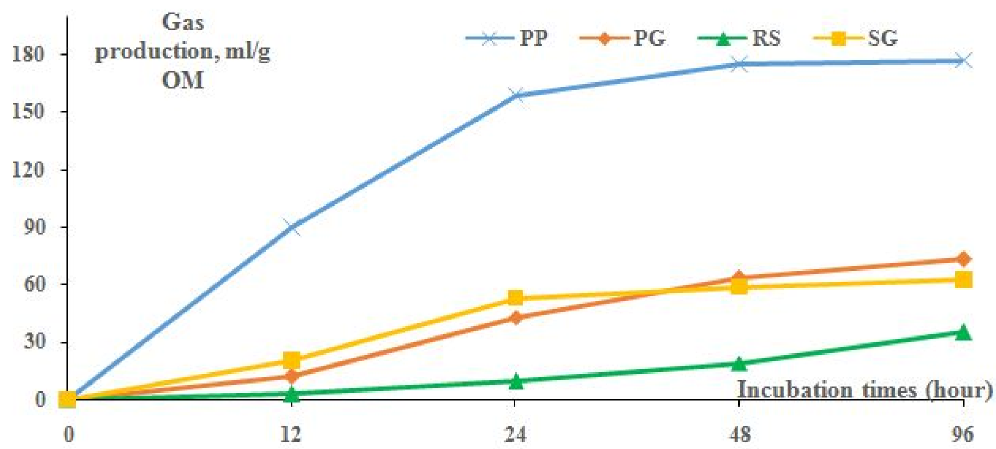

Fig 2. *In vitro* gas production (ml/gOM) from feeds over incubation times

The *in vitro* gas production curves of feeds were presented in Fig 2. It is also showed that the accumulated gas production was sharply increased from 0 to 24 hours in different feeds, while it was slightly increased from 24 to 96 h (Fig. 2). Especially, gas production of pineapple peel was the highest value and surpassed compared to other feeds. Because pineapple peel had the highest NFE content (72.1%), while this was lower for *Sesbania grandiflora* and Para grass (50.0 and 45.8%, respectively). Lee et al. (2003) also demonstrated that NFE was the most important factor in gas and methane production. Rice straw generally had lower nutrients compared to the other ones, and then its gas production was the lowest value. In summary, feeds are rich in nutrients, microorganisms could ferment and dissolve very quickly and strongly, and as a result the gas produced would be more. In contrast, poor nutritional feeds slowly and weakly demonstrated in gas production development. Similar results were also found and indicated by Paya et al. (2007) and Olfaz et al. (2018).

CONCLUSIONS

It was concluded that:

Five faecal sources in the present study could be used as inocula for the *in vitro* gas production technique and they differently affected on *in vitro* gas production.

Goat faeces used as inoculate could produce the highest gas production compared to the others, while pineapple peel used as substrate gave the highest gas production.

Studies of different faeces as inocula sources compared to rumen fluid for *in vitro* gas production should be considered to mitigate the use of rumen fluid, which are directly taken via oral esophagus tube or canula of fistulated ruminants dealing with the animal welfare.

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