Investigation On The Potential Of *In Vitro* Gas Production Technique For Assessing The Nutritive Value Of Feeds Consumed By Asian Elephants

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Abstract : The intricacies in the digestion and metabolism of nutrients are less understood and it is very difficult to assess the nutritive value of feed offered to elephants in wild as well as in captivity. Elephant being a hind gut fermenter, a novel technique was adopted to investigating the potentiality of using *In vitro* gas production technique (IVGPT) to study the digestibility of organic matter and metabolizable energy content in the feed fed to elephants under captivity using elephant dung as inoculum. IVGPT was carried out as per standard method suggested by Menke *et al*., 1979. The gas production of samples was recorded after 24, 48 and 72 hours of fermentation. *In vitro* organic matter digestibility and metabolizable energy obtained was higher for concentrates and lower for roughages. *In vitro* organic matter digestibility and metabolizable energy respectively of grass, palm leaves, straw, jack leaves, plantain stem, maize and ragi were 25.74, 28.60, 14.72, 27.95, 30.54, 67.87 and 55.37 per cent, respectively and 3.75, 4.15, 2.08, 4.06, 4.52, 10.26 and 8.35 MJ/kg DM respectively. Further studies are necessary to evaluate the efficiency of utilizing elephant dung as source of inoculum to predict nutritive value of feeds fed to elephants.

Keywords *Invitro* gas production, Elephant dung, *Invitro* organic matter digestibility.

I. INTRODUCTION

In vitro gas production test (IVGPT) is a simple and economical method to predict the energy value of feeds and fodders and digestibility when compared to slow and costly *In vivo* methods. IVGPT is mainly conducted in ruminants using rumen fluid as inoculum. Macheboeuf *et al*. (1998) predicted organic matter digestibility of forages in horses using horse faecal inoculum. Considering this, *In vitro* gas production tests using elephant dung may help to predict the energy values of feeds and fodder since, elephants are also hind gut fermenters like horses and digestive physiology is also similar. Even many of the nutritional requirements of elephants are directly extrapolated from horse's dietary requirements (Ullrey *et al.*,1997). This study was conducted to understand whether, elephant dung can be used as the source of inoculum to conduct *In vitro* gas production test and thereby predict the organic matter digestibility of feed and fodder.

II. MATERIALS AND METHODS

Samples of feeds and fodder that are generally been consumed by the elephants like maize grain, ragi grain, palm leaves, grass, plantain stem, jack leaves and straw were taken and analyzed for the proximate principles as per standard procedure (AOAC, 2016).

Concentrate feed and forage samples used in the study were ground at 1-mm particle size and 200 mg sample were taken and introduced into calibrated glass syringes (100 ml). The pistons were lubricated with vaseline. Elephant dung was collected soon after voiding and after removing the external layer, faecal sample was collected in a pre-warmed flask containing warm water at 39˚ C. The buffer solution was prepared as described by Menke and Steingass (1988). Fresh faeces (600 g) were blended with 1.2 litres of buffer solution in a mixer for 3 minutes and the faecal fluid was filtered through four layers of cheese cloth into a warm flask (39˚C). The syringes were then incubated at 39˚C with 30 ml fermentation medium. All procedures were done under anaerobic conditions. The gas production of samples was recorded after 24, 48 and 72 hours of fermentation. The gas production was measured in triplicate for blanks and the feed samples.

In vitro organic matter digestibility of feed stuffs was estimated using Eq. 1 and Eq. 2, suggested by Menke *et al*., 1979.

Eq. 1: Organic matter digestibility (per cent),

for roughages feeds = 15.38+0.8453 GP + 0.0595* P + 0.0675*TA

for concentrate feeds = 14.88 + 0.8893 GP + 0.0448 * P + 0.0651*TA

Eq. 2: Metabolizable energy (MJ/kg DM),

for roughage feeds = 2.20+0.1357*GP+0.0057*CP+0.0002859*EE,

for concentrate feeds = 1.06+0.157*GP+0.0084*CP+0.022*EE-0.0081*TA,

where, GP = gas produced (ml) in 72 h, P = crude protein content of feed, TA= ash content of feed.

Statistical analysis of the data obtained was done using independent t test to check whether there is any significant difference in gas production between concentrates and roughages

III. RESULTS AND DISCUSSION

In this study, elephant faeces were used as an inoculum in order to understand *In vitro* gas production, metabolizable energy content and the *In vitro* organic matter digestibility**.** The proximate composition of the feed samples is given in Table 1.

Gas production was recorded after 24, 48 and 72 hours of incubation. In case of roughages, gas production was negative in the first 24 hours and increased thereafter. Gas produced from roughages reached maximum by 48-72 hours of incubation, whereas, gas production from concentrates peaked in the first 24 hours of incubation. Amount of gas produced was maximum for maize, followed by ragi, palm leaves and least for straw. The results obtained is summarised in Table 2. There was a significant lag period for the amount of gas produced from roughage to peak up when compared to that of concentrates. The negative values obtained for gas production in the initial 24h may be because of antinutritional factors in roughages which could inhibit the microbial population from fermenting the feed stuff. The increased amount of gas produced from concentrates suggest that the microorganisms in elephant hindgut is more capable of utilizing concentrates when compared to roughages. There was a significant difference in total gas production between concentrates and roughages after 24h, 48h and 72h of gas production ($p<0.05$) using elephant dung as inoculum.

For the calculation of *in vitro* organic matter digestibility, total gas produced for 72 hr was considered, since the feed stuffs were not subjected to any sort of treatments, prior to incubation. *In vitro* organic matter digestibility and metabolizable energy respectively of grass, palm leaves, straw, jack leaves, plantain stem, maize and ragi were 25.74, 28.60, 14.72, 27.95, 30.54, 67.87 and 55.37 per cent respectively, and 3.75, 4.15, 2.08, 4.06, 4.52, 10.26 and 8.35 MJ/kg DM, respectively. The *In vitro* organic matter digestibility calculated from 72h of gas production of roughage was lower than the *In vivo* organic matter digestibility coefficients obtained from Asian elephants fed with grass hay by Benedict (1936) (44.6 per cent) and Foose (1982) (46.4 per cent). ICAR (2013) mentioned the *In vivo* apparent digestibility of organic matter for hay only diet, hay plus pelleted feed-based diet, hay plus oat-based diet and hay plus beet- based diet in Asian elephants as 35.53, 38.25, 38.16 and 35.1 per cent, respectively and the *In vitro* organic matter digestibility of roughages obtained in this study was comparable with the *In vivo* organic matter digestibility values conducted in different studies.

The metabolizable energy values of feed ingredients obtained were lower than the values obtained by Garg *et al.* (2012) using rumen fluid as inoculum in the *In vitro* studies in ruminants.

Table 1. Proximate composition of feed stuffs

Ingredients	Moisture (per cent)	Crude protein* (per cent)	Ether extract* (per cent)	Crude fibre* (per cent)	Total ash* (per cent)	Nitrogen free extract (per cent)
Grass	76.75	10.23	2.19	28.73	9.45	49.40
Jack leaves	54.37	15.46	5.29	8.13	12.51	58.61
Palm leaves	68.88	7.69	1.18	37.06	8.55	45.52
Plantain stem	91.68	2.54	1.56	21.77	6.65	67.48
Paddy straw	11.02	3.76	1.43	31.45	13.92	49.44
Maize	11.16	8.12	4.31	2.98	1.93	82.66
Ragi	8.92	6.47	2.57	4.03	2.73	84.20

*Values expressed on dry matter basis

Table 2. *In vitro* organic matter digestibility and metabolizable energy of feed stuffs

Feed	Net gas production (ml)			In vitro organic matter digestibility* (per cent)	Metabolizable energy[1] (MJ/kg DM)
	After 24 h	After 48 h	After 72 h		
Roughages					
Grass	2	4	11	25.74	3.75
Palm leaves	2	9	14	28.60	4.15
Straw	1	2	3	14.72	2.08
Jack leaves	1	9	13	27.95	4.06
Plantain stem	11	14	17	30.54	4.52
Concentrates					
Maize	53	58	59	67.87	10.26
Ragi	44	45	45	55.37	8.35

IV. CONCLUSION

This study was conducted to know whether elephant dung could be used as inoculum for IVGPT. The metabolizable energy values of feeds ingredients were lower than the values obtained using rumen fluid as inoculum in other studies. *In vitro* organic matter digestibility obtained was higher for concentrates and lower for roughages. Further studies are necessary for validation of these results and possible extension of this work in wild hind gut fermenters.

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