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DETERMINATION OF DIGESTIBILITY IN WILD UNGULATES BY IN VITRO GAS PRODUCTION TECHNIQUE USING THEIR DUNG AS INOCULUM

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ABSTRACT

A study was conducted to develop a method for nutritive evaluation of feeds and fodders given to wild ungulates at the State Museum and Zoo, Thrissur, Kerala. Dung of wild ungulates were used as inoculum sources for *in vitro* gas production technique and compared with dung and rumen inoculum of control animal (cattle). The *in vitro* organic matter digestibility (IVOMD) of green gram, bengal gram, cotton seed, pellet feed, green grass and jackfruit leaves ranged from 59.26±0.27 to 72.35±0.70, 62.90±0.04 to 70.72±0.01, 34.59±0.69 to 37.94±0.14, 47.74±0.0 to 50.29±0.50, 40.20±0.19 to 43.53±0.19 and 43.75±0.05 to 45.22±0.03 per cent, respectively in different wild ungulates such as sambar deer, hog deer, black buck and spotted deer and control animal under this study. It can be concluded that the organic matter digestibility of wild ungulates can easily be predicted by *in vitro* gas production technique (IVGPT) employing dung as a substitute source of rumen fluid inoculum.

Keywords: Faecal inoculum, IVGPT, IVOMD, Wild ungulates

INTRODUCTION

Feeding patterns of wild ungulates are diverse in the forest, which directly accounts for their health and wellbeing. Kerala has a significant number of wild ungulates in zoological parks and their feeding patterns under captivity are different from that of the wild. Hence nutritive evaluation of feeds given to wild ungulates under captivity is important for formulation of ration for wild ungulates. *In vivo* digestibility study is an important methodology for evaluation of feeds but it is very difficult to be conducted in wild ungulates. A simple and effective method to estimate the digestibility of feeds and fodders is *In vitro* gas production technique

(IVGPT) where the rumen liquor or dung is used as inoculum. Though IVGPT studies using rumen liquor as inoculum are plenty, research works using dung are very scarce.

So, this research study is designed to evaluate IVGPT using dung as inoculum and to assess the nutritive value of feeds offered to Sambar deer (*Rusa unicolor*), Hog deer (*Hyelaphus porcinus*), Black buck (*Antelope cervicapra*) and Spotted deer (*Axis axis*) at Zoological Park, Thrissur, by IVGPT.

MATERIALS AND METHODS

A survey was conducted to understand the feeds and fodder given to wild ungulates under captivity at State Museum and Zoo, Thrissur. Feed samples such as green gram (*Vigna radiata*), bengal gram (*Cicer arietinum*), corticated cottonseed (*Gossypium sp*.), pelleted feed and fodder, given to wild ungulates were collected from State Museum and Zoo, Thrissur and analysed for proximate principles as per standard procedure (AOAC, 2016).

Rumen liquor was collected from a five-year-old female Holstein Friesian crossbred cattle maintained on a standard ration in the University Livestock Farm and Fodder Research and Development Scheme, Mannuthy using a stomach tube after two hours of morning feeding. The rumen liquor was then transferred into a pre warmed thermos flask, strained through a four layered muslin cloth and was used as control rumen inoculum.

Fresh dung samples (100g) from six animals each, from sambar deer, hog deer, black buck, spotted deer and 600g fresh dung sample from the control animal were collected manually as and when it was voided in a container which was maintained at 39 ºC. Immediately after collecting the dung samples, the container was filled with carbon dioxide gas to maintain the anaerobic condition. The collected faeces were transported within 30 minutes to the Animal Nutrition Laboratory, CVAS, Mannuthy.

The feed substrates were air dried and ground to pass a one mm sieve and transferred 200 mg weighed substrates to the bottom of 100ml calibrated glass syringes. Faecal inoculum was prepared by combining the faecal sample with buffer solution in a ratio of 1:2 (mass/volume) under continuous flushing with CO□ gas.

The feed samples were subjected to *in vitro* trials according to the procedure described by Menke and Steingass (1988) to estimate various rumen fermentation parameters such as total gas production and *in vitro* organic matter digestibility. With

every batch of incubations, three syringes were kept as blank (without feed stuff), three syringes for concentrate reference standard and three syringes for roughage reference standard. The gas production of samples was recorded after 24, 48 and 72 hours of fermentation. Corrected net gas production was calculated by applying correction factor depending on the gas production of roughage standard and concentrate standard.

RESULTS AND DISCUSSION

In vitro gas production technique was evolved to assess the nutritive value of ruminant feedstuffs by employing fermentation technique. When a feedstuff is incubated with buffered rumen inoculum or faecal inoculum, it undergoes degradation and fermentation results in production of volatile fatty acids and gases or microbial biomass. The chemical composition of feedstuffs, corrected net gas production and IVOMD of feeds and fodders in different sources of inoculum are presented in table 1, 2 and 3, respectively.

The IVOMD of concentrate feeds ranged from 37.82±0.41 to 70.95±0.59, 37.94±0.14 to 72.35±0.70, 66.32±1.17 to 68.10±1.08 and 34.59±0.69 to 70.49±0.32 per cent in sambar deer, hog deer, black buck and spotted deer, respectively. Among all the wild ungulates under this study hog deer showed a higher IVOMD for all the concentrate and roughage feeds. This is in agreement with Li *et al*. (2018), who reported that *Succini vibrio* sp. and *Eubacterium* sp. were abundant in faecal pellets of *Axis porcinus* (11.58 and 0.028 per cent, respectively), but were hardly noticeable in red deer, sika deer, sambar deer, fallow deer, Père David's deer and tufted deer. Because of greater potential metabolic capacity of *Succini vibrio* sp. and *Eubacterium* sp., viz., syntrophic interactions with methanogens and propionate synthesis, hog deer might have better digestion efficiency than other wild ungulates under this study.

The *in vitro* organic matter digestibility of green gram in control animal were significantly lower ($p<0.05$) from all wild ungulates under study, while there was no significant difference ($p>0.05$) between faecal and rumen inoculum of the control animal. The IVOMD of green gram in black buck faecal inoculum was significantly lower ($p<0.05$) than that of other Cervidae species.

The IVOMD in percentage, for bengal gram was highest in faecal inoculum of hog deer (70.72± 0.01), followed by sambar deer (69.27±0.56), spotted deer (68.22±0.07), black buck (68.10±1.08), in descending order. The rumen inoculum of control animal (64.40±0.80) and faecal inoculum of control animal (62.90±0.04)

where in the same order as IVOMD of green gram. The IVOMD of bengal gram in sambar deer and hog deer were consistent with the findings of Ayasan *et al*. (2018), who used sheep rumen fluid inoculum and got IVOMD of 68.69 to 91.64 per cent across eight cultivars of Bengal gram.

The IVOMD in percentage, of cotton seed in the rumen inoculum (35.94±0.00) and faecal inoculum (34.99±0.11) of control animal did not show any significant difference ($p>0.05$), and were significantly lower ($p<0.05$) than the faecal inoculum of hog deer (37.94±0.14) and sambar deer (37.82±0.41). However, the IVOMD of cotton seed in the present study was lower than the value observed by Hahm *et al*. (2013), who got IVOMD of 52.61 per cent for whole cotton seed by using cattle rumen liquor inoculum.

Among the different species under this study, faecal inoculum of spotted deer (50.29±0.50 per cent) showed greater IVOMD for pellet feed, while hog deer and black buck weren't fed with pellet. Interestingly, there was no significant difference ($p>0.05$) in IVOMD of pellet feed between the control and experiment animals.

The IVOMD of all concentrate feeds were higher in wild ungulates compared to the control animal. It might be due to longer retention time of feed particles in caecum and colon of wild ungulates compared to cattle (Asano *et al*., 2005).

In the present study, rumen inoculum of control animal had highest IVOMD for green grass (43.53±0.19 per cent) and jack leaves (45.22±0.03 per cent) compared to wild ungulates, and black buck faecal inoculum (40.20±0.19 per cent) showed lowest IVOMD for green grass. Whereas the lowest IVOMD for jack leaves was observed in spotted deer (43.75±0.05 per cent). Using cattle rumen fluid inoculum, Aganga and Tshwenyane (2004) observed IVOMD of 56.9 to 87.7 per cent over different cultivars of guinea grass, which were higher than the results reported here.

The higher IVOMD of control animal for roughage sources over the sambar deer, hog deer, black buck and spotted deer might be due to the greatest ability of grazers to digest the structural carbohydrates of plant cell wall compared to intermediate feeders and concentrate selectors. The fermentation in grazers were predominated by cellulolytic bacteria, while amylolytic bacteria are were more in fermentation of concentrate selectors (Hofmann, 1989).

CONCLUSION

This study revealed that *in vitro* gas production technique can be used as a

Table 1: Chemical composition (%, on dry matter basis) of feeds offered to wild ungulates

Nutrients	Green gram	Bengal gram	Cotton seed	Pellet feed	Grass	Jack leaves
DM	97.76	97.49	96.12	96.07	24.59	43.23
CP	22.83	19.51	30.12	20.06	20.26	17.06
EE	1.06	5.83	24.35	2.02	3.41	2.87
CF	1.28	0.90	10.35	10.11	33.53	16.62
Total ash	3.53	2.62	5.42	12.15	13.73	9.67
NFE	71.28	71.12	29.74	55.64	29.07	53.76

Table 2: Corrected net gas production of evaluated feeds and fodder (mL/200mg DM) after 72 hrs incubation in different wild ungulates (Mean±SE)

Feed ingredients	Control animal RL	Control animal faeces	Sambar deer	Hog deer	Black buck	Spotted deer
Green gram	49.04±1.26	48.39±0.30	61.54±0.67	63.12±0.79	56.34±1.31	61.02±0.36
Bengal gram	54.43±0.90	52.74±0.04	59.90±0.63	61.53±0.01	58.59±1.22	58.72±0.08
Cotton seed	21.64±0.00	20.57±0.12	23.75±0.46	23.89±0.16	Not given	20.12±0.78
Pellet feed	37.03±1.65	36.16±0.53	34.96±0.00	Not given	Not given	37.83±0.56
Green grass	30.81±0.23	29.66±0.21	27.76±0.53	29.07±0.42	26.88±0.22	28.80±0.11
Jack leaves	33.36±0.04	33.18±0.06	32.04±0.81	32.13±0.28	31.71±0.26	31.61±0.06

Table 3: *In vitro* organic matter digestibility (per cent) * of evaluated feeds and fodder in different wild ungulates (Mean±SE)

Feed ingredients	Control animal RL	Control animal faeces	Sambar deer	Hog deer	Black buck	Spotted deer
Green gram	59.83 ± 1.12^{a}	59.26 ± 0.27^{a}	70.95 ± 0.59^{b}	72.35 ± 0.70^{b}	66.32 ± 1.17^{c}	70.49 ± 0.32^{b}
Bengal gram	64.40 ± 0.80^{a}	62.90 ± 0.04^{a}	69.27 ± 0.56^{b}	70.72 ± 0.01^{b}	68.10 ± 1.08^{b}	68.22 ± 0.07^{b}
Cotton seed	35.94 ± 0.00^{a}	34.99 ± 0.11^{a}	37.82 ± 0.41^{b}	37.94 ± 0.14^{b}	Not given	34.59 ± 0.69^{a}
Pellet	49.58 ± 1.47^{a}	48.80 ± 0.47^{a}	47.74 ± 0.0^{a}	Not given	Not given	50.29 ± 0.50^{a}
Grass	43.53 ± 0.19^{a}	42.58 ± 0.18^{ac}	40.95 ± 0.45^{bd}	42.06 ± 0.35^{bc}	40.20 ± 0.19^{d}	41.83 ± 0.10^{bc}
Jack leaves	45.22 ± 0.03^{a}	45.09 ± 0.05^{ab}	44.11 ± 0.68^{ab}	44.18 ± 0.23^{ab}	43.83 ± 0.22^{ab}	43.75 ± 0.05^{b}

Means bearing different superscripts in a row differ significantly at 5 per cent significance level. *Organic matter digestibility, for roughage 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

Where, GP = corrected net gas production, P = crude protein content of feed, TA= ash content of feed

simple method for calculating the *in vitro* organic matter digestibility of feed and fodder given to wild ungulates by using their dung as an inoculum source, since it is difficult to conduct an *in vivo* digestibility study on zoo animals especially which are reared on group feeding. It also points out that faecal microbiota among different wild ungulates differ significantly irrespective of the nature of the diet, which in turn results in different fermentation parameters for the same feed.

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