



# IN VITRO EVALUATION OF TREE FODDERS AS AN ALTERNATE FEED SOURCE USING –IVGPT

Sajith Purushothaman<sup>1</sup>, K. Shyama<sup>2</sup>,  
K. Ally<sup>3</sup>, V. Dildeep<sup>4</sup>, K. Jasmine Rani<sup>5</sup> and  
C. Sunanda<sup>6</sup>

Department of Animal Nutrition, College of  
Veterinary and Animal Sciences,  
Mannuthy – 680 651, Thrissur, Kerala.

Received: 02.08.2018  
Accepted: 13.10.2018

## Abstract

The study was conducted to evaluate the effect of inclusion of tree fodder as cattle feed ingredient in TMR. Four TMRs were prepared by using tree fodders such as gliricidia leaves (*Gliricidia sepium*), moringa leaves (*Moringa oleifera*), desmanthus (*Desmanthus virgatus*) and jack leaves (*Artocarpusheterophyllus*) replacing the conventional ingredients on the basis of chemical composition. *In vitro* gas production technique (IVGPT) was done for evaluation of the TMR. The concentrate to roughage ratio of the ration was maintained as 50:50. The proximate analysis revealed that tree fodder can be incorporated in cattle TMR both as protein and fibre source. IVGPT results indicated that the *in vitro* dry matter digestibility, organic matter digestibility and microbial bio mass production was maximum in TMR-1 (TMR containing gliricidia leaves). Methane production was seen minimum in TMR-4. Among the four TMRs tested TMR-1 containing Gliricidia produced best results and hence can be incorporated in cattle TMR and

hence it can be concluded that Gliricidia leaves can be incorporated in the cattle ration with improved rumen fermentation potential

**Key words :** IVGPT, Gliricidia, TMR

The feeding cost increased to about 80 per cent on last decade due to the shortage of feed grains. In Kerala there is a huge gap between feed and fodder requirement and availability (Economic Review, 2017). To bridge the gap between the availability and requirement of feed some alternate feed sources has to be tried. The unconventional fibre source includes various tree fodders which can contribute good quality and quantity of protein. Tree fodders are popular among the livestock farmers as fodder source in scarcity periods. Plant secondary metabolites and tannic acid are believed to have an ameliorative effect on methane emission as well. It also possess many positive effects such as improved daily gain, increased voluntary feed intake, protective effect on protein utilization and

1, 4, 5 & 6. Assistant Professor

2. Associate professor, Department of Animal Nutrition, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala.

3. Professor and Head

6. Assistant Professor, Department of Statistics, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala.

Corresponding author Email – sajith@kvau.ac.in Ph No - 9447019183

decreasing parasitic load in animals (Peng *et al.*, 2016). The major problem in feeding is the deleterious effect caused by plant secondary metabolites such as tannin, saponins and other toxic factors. Excess feeding may cause very deleterious effect on animal health. Hence proper processing and level of incorporation has to be fixed carefully. Evaluating nutritive value of unconventional feed ingredients has to be done prior to incorporation in animal feeds to study the rumen fermentation pattern. *In vitro* gas production technique can be used as a perfect tool for this (Mould *et al.*, 2005). The assumption that *in vitro* results will mimic the fermentation characteristics like substrate digestion, volatile fatty acid production, nutrient digestibility and microbial protein production as in *in vivo* conditions (Keiser and Weniger, 1994).

Total mixed ration (TMR) feeding helps in stabilization of rumen fermentation and ensures better ammonia utilization and help in incorporating poor quality roughage and unconventional feed stuffs. TMR feeding will also ensure the sufficient concentrate: roughage in lactating animals, since selection among feed is not possible. Influence of unconventional feeds on rumen microbes and fermentation pattern are not much studied. Hence the study has been taken up with following objectives.

1. Evaluation of total mixed ration containing various unconventional feed ingredients by *in vitro* gas production technique.
2. To suggest suitable total mixed ration for crossbred cattle for efficient nutrient utilization

## Materials and Methods

### Sample collection and preparation

The tree fodders such as, *Gliricidia sepium*, *Moringa oleifera*, *Desmanthus virgatus* and *Artocarpus heterophyllus* were collected locally. The samples were dried and ground to pass through a 1mm screen and their proximate analysis was done as per AOAC (2012) (Table-2). Four iso caloric and iso nitrogenous total mixed rations were prepared by using these unconventional feed ingredients

replacing conventional ingredients from concentrate mixture. The proximate analysis of all the four TMRs were done as per standard procedure AOAC (2012) (Table 3). The ingredient composition of four TMRs prepared was given in Table-1

### *In Vitro* Gas Production Technique

The four TMR's were subjected to *in vitro* trials according to the procedure described by Menke and Steingass (1988). Rumen liquor was collected from six crossbred cows maintained in standard farm ration using a stomach tube before morning feeding. The rumen liquor was transferred into a pre warmed thermos flask and strained through a four layered muslin cloth and pooled together which was used as inoculum for conducting *in vitro* trial to estimate various rumen fermentation parameters.

### Total Gas Production

Gas produced (ml/ 200 mg substrate) by fermentation of substrate feed during 24 hour was measured after correcting corresponding blank values Menke and Steingass, (1988).

### *In vitro* True DM AND OM Digestibility

Goering and Van Soest (1970) method was followed for the determination of true DM and OM digestibility of TMR used as substrate

$$\text{TDMD\%} = (\text{DM taken for incubation} - \text{NDF residue}) \times 100$$

(DM taken for incubation)

$$\text{TOMD\%} = (\text{OM taken for incubation} - \text{residual OM}) \times 100$$

(OM taken for incubation)

### Microbial Biomass Production (MBP)

Microbial biomass production (MBP) of the TMR tested was calculated from TDOM using equation

$$\text{MBP (mg)} = \text{TDOM (mg)} - (\text{Corrected gas production for 24 hrs} \times 2.20)$$

Where 2.20 is the stoichiometric factor for roughages (Blummelet *et al.*, 1997) and for mixed diets (Blummel and Lebzien 2001).

**Metabolizable Energy (ME)**

ME of target TMR was calculated by the method of Menke and Stienlisset *al.* (1988)

$$\text{ME (kJ/kg DM)} = 1.24 + 0.146 \times \text{gas (ml/200mg DM)} + 0.007 \times \text{CP} + 0.0224 \times \text{EE}$$

Where, CP - crude protein, EE - ether extract

TA- Total Ash, GP- corrected gas production for 24 hours.

**Methane Estimation**

Methane production capacity of the TMR was determined by using methane sensor fabricated analyzer developed in Kerala Veterinary and Animal Sciences University. The data from the experiment were analyzed statistically as per Snedecor and Cochran (1994).

**Results and Discussion**

The analysis of chemical composition of gliricidia leaves, moringa leaves, desmanthus and jack leaves revealed that the moisture was 59.58, 77.50, 71.70 and 59.75 per cent respectively. The crude protein values were 21.68, 17.80, 22.66 and 16.54 per cent, ether extract values were 11.46, 5.10, 2.29 and 1.63 per cent, crude fiber values were 24.14, 15.70, 22.50 and 18.37 per cent, total ash values were 10.00, 10.60, 4.37 and 9.09 per cent and nitrogen free extract were 32.24, 50.80, 48.18 and 54.37 per cent respectively for gliricidia, moringa leaves, desmanthus and jack leaves.

**Table-1: TMR-1**

Ingredient	Quantity (parts per quintal)
Maize	30
Bajra	9
Gliricidia leaves	5
Soya bean meal	21
Wheat bran	16
De oiled rice bran	14.5
Mineral mixture	3
Salt	1.5
Total	100

Similar results were reported by Aye and Adegun (2013).

The chemical composition of four TMR prepared were in Table-3 and the fermentation parameters estimated for the various TMRs were presented in Table-4

**TMR-2**

Ingredient	Quantity (parts per quintal)
Maize	30
Bajra	9
Moringa leaves	5
Soya bean meal	23
Wheat bran	16
De oiled rice bran	12.5
Mineral mixture	3
Salt	1.5
Total	100

**TMR-3**

Ingredient	Quantity (parts per quintal)
Maize	30
Bajra	9
Desmanthus	5
Soya bean meal	21
Wheat bran	16
De oiled rice bran	14.5
Mineral mixture	3
Salt	1.5
Total	100

**TMR-4**

Ingredient	Quantity (parts per quintal)
Maize	30
Bajra	9
Jack leaves	5
Soya bean meal	21
Wheat bran	18
De oiled rice bran	12.5
Mineral mixture	3
Salt	1.5
Total	100

**Table-2:** Chemical composition in % DM basis

Un conventional feed	Moisture	Crude protein	Ether extract	Crude fiber	Total ash	Nitrogen free extract
Gliricidia	59.58	21.68	11.46	24.14	10.00	32.24
Moringa leaves	77.50	17.80	5.10	15.70	10.60	50.80
Desmanthus	71.70	22.66	2.29	22.50	4.37	48.18
Jack leaves	59.75	16.54	1.63	18.37	9.09	54.37

**Table-3:** Chemical composition of TMR in % DM basis

Ingredient	DM	CP	EE	CF	NDF	ADF	Total ash	AIA	NFE	OM
TMR-1	94.07	15.79	1.45	18.50	38.89	23.26	10.7	3.95	53.36	89.30
TMR-2	93.28	15.82	1.47	18.84	42.04	24.11	10.69	4.00	53.18	89.31
TMR-3	93.39	15.18	1.50	19.00	47.58	25.49	11.19	3.65	53.13	88.81
TMR-4	93.87	15.79	1.42	18.65	42.14	25.35	11.00	3.38	53.14	89.00

**Table-4:** Fermentation parameters of TMRs using IVGPT

Parameters	TMR-1	TMR-2	TMR-3	TMR-4	p-value
Gas production (ml/200mg)	15 <sup>c</sup> ± 0.58	15 <sup>c</sup> ± 0.29	18 <sup>b</sup> ± 0.29	20 <sup>a</sup> ± 0.58	< 0.001
IVDMD%	78 <sup>a</sup> ± 0.29	71.5 <sup>c</sup> ± 0.12	69.5 <sup>d</sup> ± 0.29	72.5 <sup>b</sup> ± 0.29	< 0.001
IVOMD%	77.41 <sup>a</sup> ± 0.12	70.99 <sup>b</sup> ± 0.23	71.61 <sup>b</sup> ± 0.73	70.09 <sup>b</sup> ± 0.6	< 0.001
ME (kJ/kgDM)	4.78 <sup>c</sup> ± 0.01	4.78 <sup>c</sup> ± 0.01	5.32 <sup>b</sup> ± 0.02	5.5 <sup>a</sup> ± 0.01	< 0.001
Microbial biomass production (mg)	44.41 <sup>a</sup> ± 0.01	37.99 <sup>b</sup> ± 0.01	32.26 <sup>c</sup> ± 0.01	25.59 <sup>d</sup> ± 0.01	< 0.001
Methane %	8.9 <sup>a</sup> ± 0.01	8.5 <sup>c</sup> ± 0.01	8.6 <sup>b</sup> ± 0.03	7.72 <sup>d</sup> ± 0.01	< 0.001

Means bearing different superscripts within same rows differ significantly ( $P < 0.01$ )

Among the four TMRs gas production was significantly higher in TMR-4 i.e.  $20 \pm 0.58$  compared to TMR-1, 2 and 3 and the values were  $18 \pm 0.29$ ,  $15 \pm 0.29$  and  $15 \pm 0.58$  respectively and TMR -3 showed significantly higher gas production than TMR 1 and 2. The IVDMD and IVOMD per cent were significantly higher in TMR 1 than TMR 2, 3 and 4. The values for IVDMD (%) in TMR 1, 2, 3 and 4 were  $78 \pm 0.29$ ,  $71.5 \pm 0.12$ ,  $69.5 \pm 0.29$  and  $72.5 \pm 0.29$  respectively. The IVOMD (%) was  $77.41 \pm 0.12$ ,  $70.99 \pm 0.23$ ,  $71.61 \pm 0.73$  and  $70.09 \pm 0.6$  respectively for TMR 1, 2, 3 and 4. Metabolisable energy values were  $4.78 \pm 0.01$ ,  $4.78 \pm 0.01$ ,  $5.32 \pm 0.02$  and  $5.50 \pm 0.01$  respectively for TMR1, TMR-2, TMR-3 and TMR-4. And the significantly higher value was observed in TMR-4 compared to TMR 3, 2 and 1. The significantly higher microbial biomass production was seen in TMR-1 i.e.  $44.41 \pm 0.01$  when compared to TMR 2, 3 and 4 and the respective values were  $37.99 \pm 0.01$ ,  $32.26 \pm 0.01$  and  $25.59 \pm 0.01$ . The minimum methane production (%) was seen in TMR- 4  $7.72 \pm 0.01$

which was significantly lower than in TMR-2, 3 and 4 and values were  $8.50 \pm 0.01$ ,  $8.6 \pm 0.03$  and  $8.9 \pm 0.01$  respectively. From the above parameters the TMRs showing maximum TDMD (%), TOMD (%), MBP (mg) were TMR-1, but TMR-4 showed higher metabolisable energy and minimum methane production. Hence it was concluded that TMR-1 containing Gliricidia leaves can be recommended among above four TMRs but the other ingredients can also be incorporated with a comparable efficiency. The results obtained are in accordance with the findings of Wahayuni *et al.* (2009) and Reddy *et al.* (2016). The higher microbial biomass production in TMR-1 indicates that the combination of feed in the ration resulted in highest nutrient rich supplementation which otherwise promote maximum microbial activity. The results are in agreement with the findings of Suharlina *et al.* (2016). The methane production was minimum in TMR-4 this is mainly attributed to the condensed tannin which inhibits the methane forming microbes for methane formation.

## References

- AOAC. 2012. Official Methods of Analysis. (19<sup>th</sup> Ed.). Association of Official Analytical Chemists, Washington, D. C. pp.1-77.
- Aye P.A and Adegun M. K.2013. Chemical composition and some functional properties of Moringa, Leucaena and Gliricidia leaf meals. *Agri.biol. J. N. Am.* **4**:71-77.
- Blummel, M. and Lebzien, P. 2001. Predicting ruminal microbial efficiencies of dairy rations by in vitro techniques. *Livest. Prod. Sci.* **68**: 107-117.
- Blummel, M., Steingass, H. and Becker, K.1997. The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and N incorporation and its implications for the prediction of voluntary feed intake of roughages. *Br. J. Nutr.* **77**: 911- 921.
- Economic review. 2017. *Agriculture and allied sectors. Chapter 2.State Planning Board, Thiruvannathapuram,Kerala. India.* pp. 27-39.
- Goering, H.K. and Van Soest, P.J. 1970. *Forage fibre analysis (apparatus, reagents, procedures and some applications)*. ARS, USDA, Washington. Agriculture Hand book. 379.
- Kaur, J. and Thakur, S.S. 2017. *In vitro* methane production potential, nutrient digestibility and fermentation pattern of total mixed rations containing incremental levels of novel feed stuff *Phalaris minor* seeds in Buffalo inoculum. *Inj. J. Curr. Microbiol. App. Sci.* **6**: 96-103.
- Kaiser.D.andWeniger,J.H. 1994. *In vivo* and *in vitro* investigation for nutrient digestibility and heat production of ruminants under heat stress and different nutritional levels.V. Comparison of in vivo and in vitro investigations with respect to energy metabolism and energy content of rations. *Archiv.Tierzucht.* **37**:535- 545.
- Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Anim. Res. Dev.* **28**: 7-55.
- Mould,F.F.,Kleim,K.E.,Morgan,R. and Mauricio,R.M.2005. *In vitro* microbial inoculums a review of its fuction and properties. *Anim. Feed Sci. Technol.* **123/124**:31-50.
- Peng,K.,Shirley,D.C.,Xu,Z.,Huang.Q.,MCallisar,T.A.,Chaves,A.V.,Acharya,S., Liu,C., Wang.S. and Wang.Y. 2016. Effect of purple prairie clover (*Dalea purpurea*) hay and its condensed tannins on growth performance, wool growth, nutrient digestibility, blood metabolites and ruminal fermentation in lambs fed total mixed rations. *Anim. Feed Sci. andTechol.* **222**: 100-110.
- Reddy, Y.R., Kumari, N.N., Monika, T. and Sridhar, K. 2016. Evaluation of optimum roughage to concentrate ratio in maize stover based complete rations for efficient microbial biomass production using *in vitro* gas production technique. *Vet. Wld.* **9**: 611-615.
- Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.*(8<sup>th</sup> Ed.). The Iowa State University press, Ames, 4p.
- Suharlina.,Astuli.D.A.,Nattrowl., Jayanegara. A. and Abdullah.L.2016. Nutritional evaluation of dairy goat rations containing *IndigoferaZollingeriana* by using *in vitro* rumen fermentation technique (Rusitec), *Int.J.of Dairy Sci.* **11**: 100- 105
- Wahayuni, D.S. Tjakradidjaya, A.S. and Suharyono. 2009. *In vitro* fermentability, degradability and microbial biomass product of complete ration containing a combination of field grass, concentrate and nutrient rich supplement. *J. Indonesian Trop. Anim. Agric.* **34**: 258-264.