SHORT COMMUNICATION
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2022.53.1.105-111

Evaluation of *in vitro* degradability of total mixed ration by synergistic supplementation of herbal extract-probiotic mixture in ruminants#

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Citation: Neenu, O., Surej, J.B., Biju, C., Senthil Murugan, S., Muhammed, E. M., Arathi, S., Sreeja, S. J., Sabarinath, M., Amrutha, A. and Merin Sneha, C.T. 2022. Evaluation of *in vitro* degradability of total mixed ration by synergistic supplementation of herbal extract-probiotic mixture in ruminants. *J. Vet. Anim. Sci.* **53**(1): 105-111. DOI: https://doi.org/10.51966/jvas.2022.53.1.105-111

Received: 26.03.2021 *Accepted: 17.04.2021* *Published: 31.03.2022*

Abstract

In Kerala, crossbred cattle feeding is restricted to grazing on low-quality forages with meagre allowances of concentrates and this necessitates the supplementation of feed additives for better utilisation of low quality feed resources. The probiotic culture of Aspergillus oryzae is widely used in ruminant nutrition because of its buffering function in stabilizing the rumen environment and stimulation of microbial activity especially fibrolytic activity. Psidium guajava leaves are antimethanogenic in action and the biological activities of these leaves include improving rumen fermentation and nutrient utilization in livestock. Hence a preliminary screening of the synergistic supplementation of different levels of A. oryzae and P. guajava leaf extracts at 0, 0.5, 0.25, and 0.125 per cent respectively in the total mixed ration (TMR) of concentrate: roughage (60:40) on dry matter(DM) basis was studied using in vitro gas production technique (IVGPT) with a view to estimate the metabolisable energy, digestible organic matter, methane production and in vitro degradable nitrogen(IVDN). The percentage of IVDN was significantly (p<0.01) higher for the treatment group supplemented with the herbal extract-probiotic mixture at 0.25% level. The percentage of methane production was significantly (p<0.01) lower for the treatment groups supplemented with the herbal extract-probiotic mixture at 0.5% and 0.25% with values of 8.42% and 10.8% respectively. The results indicated that synergistic supplementation of P. guajava leaf extract and A. oryzae probiotic culture each at 0.25% level in the TMR gave the best performance in terms of methane mitigation and optimum availability of IVDN for improved growth performance in crossbred dairy calves.

#Part of MVSc. thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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Keywords: *IVGPT, Aspergillus oryzae, Psidium guajava, TMR*

In Kerala, a huge gap exists between the requirement and availability of feed and fodder (Economic Review, 2017). The low-quality roughages used for feeding crossbred dairy calves are rich in neutral detergent fibre (NDF) but deficient in crude protein which may result in reduced growth and productivity. Hence, manipulation of rumen fermentation by supplementation with feed additives is essential to improve the utilization of the available feed resources and to increase the productivity of crossbred dairy animals.

Aspergillus oryzae is one of the most efficient fungal feed additives used in ruminant nutrition because of its rumen buffering activity that is essential for adequate functioning of fibrolytic bacteria and counteracting the ill effects of ruminal acidosis. That apart, it promotes the microbial degradation of other nutrients by the production of growth factors such as amino acids and B vitamins. Since the crop residues and green fodder used for feeding crossbred dairy calves are rich in crude fibre content, the addition of *A. oryzae* in feed improves fibre digestion especially cellulose and this would ensure enhanced animal productivity.

Methane emissions represent a gross energy loss of up to 15 per cent for forage-fed cattle and 2–4 per cent for cattle that feed on readily fermentable substrates (Bhatta *et al.,* 2012). Methane is also a greenhouse gas that contributes to global warming. Condensed tannins present in tropical plants such as guava (*Psidium guajava*) leaves were found to reduce methane and ammonia production. India is a major producer of guava (*P. guajava)* and the leaves of this plant have high condensed tannin content that could be harnessed for use as a feed additive at optimum levels for ruminants. However, very few studies have been conducted to study the combined effect of tannin-rich herbal extracts and probiotic cultures as feed additives in the feeding of crossbred dairy calves *in vivo* as well as *in vitro*. Hence an experiment was designed to study the synergistic effect of supplementation of dried *A. oryzae* culture and aqueous leaf extract powder of *P. guajava* as feed additives in TMR on DM basis by *in vitro* gas production technique (IVGPT) with the following objectives:

1) To evaluate the *in vitro* fermentation and *in vitro* degradability on supplementation of varied levels of *Aspergillus oryzae* and *Psidium guajava* leaves extract in TMR on DM basis by IVGPT.

2) To identify the best synergistic dose of dried *A. oryzae* culture and aqueous leaf extract powder of *P. guajava* as feed additives in TMR on DM basis for further *in vivo* study in crossbred dairy calves.

Sample collection and preparation

Isocaloric and isonitrogenous total mixed rations were prepared using commercially available Type II Cattle Feed and Hybrid Napier CO3 green fodder. These ingredients were oven-dried at 70°C and finely ground in Wiley mill using a 1 mm sieve. Concentrate and roughage were mixed in the ratio 60:40 and a total mixed ration (TMR) was prepared. The dried culture of *A. oryzae* (1 billion Colony Forming Units (CFU)/g was procured from M/s Kaypeeyes Biotech Private Limited, Mysore, Karnataka, India. The powdered aqueous extract of *P. guajava* leaves was purchased from M/s Bhagvati Herbal and Healthcare Private Limited, Valsad, Gujarat, India.

In Vitro Gas Production Technique (IVGPT) was performed to evaluate the effect of *A. oryzae* and *P. guajava* supplementation on rumen fermentation parameters, nitrogen degradability, and methane production at four different doses, *viz.*, 0, 0.5, 0.25, and 0.125 per cent, of incubated substrate of TMR comprising concentrate and roughage in 60:40 ratio on DM basis. The proximate analyses, per cent of calcium and phosphorus of *A. oryzae* dried culture and aqueous leaves extract powder of *P. guajava* and the four TMRs were done as per the standard procedure of AOAC (2016). Acid detergent fibre and neutral detergent fibre were estimated by the detergent method of analysis as per Van Soest *et al*. (1991).

In Vitro Gas Production Technique (IVGPT)

The four rations were subjected to *in vitro* trials according to the procedure described by Menke and Steingass (1988). The whole rumen cud was collected from three cattle sacrificed at the slaughterhouse and used as inoculum for the fermentation. The cattle were not sacrificed specifically for this experiment but slaughtered for meat purpose. The rumen cud was transferred into a pre-warmed thermos flask and strained through a four-layered muslin cloth and used as inoculum with continuous bubbling of carbon dioxide gas for conducting the *in vitro experiments* to estimate various rumen fermentation parameters. The per cent inclusion of different levels of *Aspergillus oryzae* culture and *Psidium guajava* leaves extract powder in the total mixed ration (TMR) on DM basis used for *in vitro* analyses by IVGPT (Menke and Steingass, 1988) are given in Table1.

Net Gas Production

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

Calculation of Metabolisable Energy (ME), Digestible organic matter (DOM), and In vitro degradable nitrogen (IVDN) content

The prediction equation for the calculation of ME and DOM from gas production data are given below:

ME (MJ/kg DM) = 1.24+ 0.146 × GP (ml/200mg DM) + 0.007× CP + 0.0224× EE

DOM (%) = 14.88+0.889 x GP (ml/200 mg DM) + 0.45 x CP+0.65 x TA

Where CP - crude protein content in %, EE - ether extract in %, TA - total ash in %, and GP - corrected gas production for 24 hours.

$$\text{IVDN (\%)} = \frac{\text{A-(A-B) C-(ammonia nitrogen of blank)}}{\text{(C-D)}} \Big/ \text{Total nitrogen of feedstuff incubated}$$

Where A - mg of ammonia nitrogen after 24 hr incubation, with no carbohydrate added; B - mg NH_3-N after 24 hr incubation with carbohydrate added; C - ml gas production in 24 hr with no carbohydrate added; D - ml of gas production in 24 hr with carbohydrate added (Raab *et al*. 1983 and Menke and Steingass, 1988).

Methane Estimation

The methane production potential of the treatment rations was determined by feeding the gas produced during the experiment into a digital methane sensor fabricated analyzer developed in Kerala Veterinary and Animal Sciences University (Purushothaman *et al.,* 2019).

All the data generated in the above experiments were statistically analysed as per Snedecor and Cochran (1994) by analysis of variance (ANOVA) technique.

Scoring system

A four-point scoring system (Raseel, 2018) as depicted in Table 2 was devised for ranking the treatment groups with different proportions of the two feed additives in total mixed ration tested in the *in vitro* study. Based on the values obtained for each of the above four parameters, individual scores were assigned for each of the groups tested and the treatment group having the highest aggregate score was selected as the best synergistic combination of the two feed additives to be used for the *in vivo* study.

The chemical composition of *A. oryzae* and *P. guajava* on a per cent DM basis is given in Table 3. The chemical composition of four total mixed rations prepared is presented in Table 4. The values obtained for the net gas production, ME, DOM, IVDN and methane production in the *in vitro* study of TMR of concentrate and roughage (60:40) containing the dried culture of *A. oryzae* and aqueous extract powder of *P. guajava* leaves in specific proportions (each at 0, 0.5, 0.25 and 0.125 per cent of TMR on DM basis) are given in Table 5.

The chemical composition of Total Mixed Ration (TMR) used for the *in vitro* study

Table 1. Per cent inclusion level of *A. oryzae* and *P. guajava* in TMR on DM basis

Treatment group	*A. oryzae* culture (%)	*P. guajava* leaves aqueous extract powder (%)
T-1 (Control)	0	0
T-2	0.5	0.5
T-3	0.25	0.25
T-4	0.125	0.125

Table 2. Score sheet for ranking the best synergistic dosage of feed additives by IVGPT:

Parameter tested *in vitro*	Unit (on DM basis)	Range of value	Score
ME	MJ/ kg DM	<4 4-5 5-6 6-7 7-8 8-9 >9	0 + ++ +++ ++++ +++++ ++++++
DOM	%	<40% 40-50 50-60 60-70 70-80 80-90 >90%	0 + ++ +++ ++++ +++++ ++++++
IVDN as % of total nitrogen	%	<40 % 40-50 50-60 60-70 70-80 80-90 >90%	0 ++++ +++++ ++++++ +++ ++ +
Methane production	%	<11% 11-20 21-30 31-40 41-50 51-60 >60%	++++++ +++++ ++++ +++ ++ + 0

was comparable with the complete rations used by Raseel (2018) for a study in lactating dairy cows. Among the four treatments, the ME ($p<0.05$) and DOM ($p<0.05$) of T-1 was significantly higher than other treatment groups. The IVDN per cent ($p<0.01$) was significantly higher for T-3 and the percentage of methane production was significantly ($p<0.01$) lower for the treatment groups T-2 and T-3. Based upon the four-point scoring system, the aggregate scores obtained for the four treatment groups, T-1, T-2, T-3 and T-4 were 9, 8, 12 and 7 respectively. Hence the treatment group T-3 supplemented with 0.25 per cent each of dried culture of *A. oryzae* and dried aqueous extract of *P. guajava*, having the highest aggregate score, was recommended as the best synergistic dosage for optimum supplementation in the basal diet of crossbred dairy calves for better rumen fermentation and nutrient utilisation.

Metabolisable Energy (ME), Digestible Organic Matter (DOM) and In Vitro Degradable Nitrogen (IVDN) Content

The results were in agreement with the findings of Hariadi and Santoso (2010) who reported that the condensed tannin present in *P. guajava* leaves interacted with feed components such as protein and carbohydrates forming hydrogen bonds. These binding of condensed tannins with carbohydrates and proteins in feed prevented its degradation and fermentation partially

Table 3. Chemical composition of the dried culture of *A. oryzae* and dried aqueous extract of *P. guajava leaves* on % DM basis

Parameters	*A. oryzae*	*P. guajava*
Dry matter (DM)	90.67 ± 0.07	93.91 ± 0.10
Organic matter (OM)	93.12 ± 0.01	99.55 ± 0.15
Crude protein (CP)	25.97 ± 0.03	14.12 ± 0.99
Crude fibre (CF)	15.64 ± 0.60	0.55 ± 0.15
Ether extract (EE)	3.54 ± 0.40	0.59 ± 0.10
Total ash (TA)	6.89 ± 0.01	0.01 ± 0.15
Nitrogen free extract (NFE)	47.98 ± 0.23	84.30 ± 1.38
Acid insoluble ash (AIA)	0.20 ± 0.00	BDL*
Neutral detergent fibre (NDF)	45.08 ± 0.39	5.12 ± 0.60
Acid detergent fibre (ADF)	22.33 ± 0.15	0.40 ± 0.20
Hemicellulose (HC)	22.76 ± 0.25	4.72 ± 0.40
Ca	0.17 ± 0.00	BDL*
P	4.70 ± 0.01	BDL*

*BDL – Below Detectable Level

Table 4. Nutrient composition of experimental TMRs used in IVGPT in % DM basis

Parameter	T-1 (0)	T-2 (0.5%)	T-3 (0.25%)	T-4 (0.125%)
DM	64.2 ± 0.11	65.12 ± 0.11	64.66 ± 0.11	64.43 ± 0.11
OM	89.62 ± 0.06	90.58 ± 0.06	90.10 ± 0.06	89.86 ± 0.06
CP	15.88 ± 0.59	16.07 ± 0.58	15.97 ± 0.58	15.92 ± 0.58
CF	22.22 ± 0.15	22.3 ± 0.15	22.26 ± 0.15	22.24 ± 0.15
EE	2.43 ± 0.06	2.45 ± 0.06	2.44 ± 0.06	2.43 ± 0.05
TA	10.39 ± 0.06	10.43 ± 0.06	10.41 ± 0.06	10.40 ± 0.06
NFE	49.1 ± 0.84	49.76 ± 0.83	49.43 ± 0.84	49.27 ± 0.84
AIA	2.54 ± 0.03	2.54 ± 0.03	2.54 ± 0.03	2.54 ± 0.03
NDF	47.75 ± 0.49	48.00 ± 0.48	47.87 ± 0.48	47.81 ± 0.48
ADF	27.48 ± 0.01	27.60 ± 0.01	27.54 ± 0.01	27.51 ± 0.01
HC	20.27 ± 0.48	20.40 ± 0.47	20.33 ± 0.47	20.30 ± 0.47
Ca	0.85 ± 0.02	0.85 ± 0.02	0.85 ± 0.02	0.85 ± 0.02
P	0.48 ± 0.03	0.50 ± 0.03	0.49 ± 0.03	0.48 ± 0.03

Average of six values with S.E.

and thus lowered the gas production causing a decreased value of DOM and ME content in substrate supplemented with *P. guajava* leaves in *in vitro* experiment, similar to the present study.

Considering the results of IVDN, Beharka *et al.* (1991) reported that low (0.09 per cent) and moderate level (0.18 per cent) of dietary supplementation of *A. oryzae* produced higher quantities of rumen ammonia concentration compared with high (0.55 per cent) level of *A. oryzae,* similar to the present study. Sosa *et al.* (2010) reported that the addition of *A. oryzae* enhanced microbial colonization in the rumen by the release of growth factors such as amino acids and B vitamins. Azzaz *et al.* (2015) reported a positive effect of *A. oryzae* on rumen protozoal count which in turn cause improved nitrogen turn over in rumen of lactating sheep.

According to Patra and Saxena (2010), tannins are water-soluble polyphenolic polymers which form complexes mainly with proteins due to the presence of a large number of phenolic hydroxyl groups. Hence, the concentration of tannin present in the aqueous extract of *P. guajava* leaves used in the present study was higher compared to that of *P. guajava* leaves as such.

The protein binding activity of condensed tannins from guava leaves with the subsequent formation of condensed tannin-protein complexes was responsible for the reduced proteolysis to yield ammonia. (Pal *et al.*,

Table 5. Fermentation parameters of TMRs using IVGPT

Parameter	T-1 (0)	T-2 (0.5%)	T-3 (0.25%)	T-4 (0.125%)	F-value	p-value
Net gas production (ml/200 mg DM)	27.86±1.88^{a}	22.41±1.14^{b}	22.47±0.83^{b}	22.86±1.62^{b}	3.45*	0.02
ME (MJ/kgDM)	5.46±0.27^{a}	4.67±0.17^{b}	4.68±0.12^{b}	4.73±0.24^{b}	3.45*	0.02
DOM (%)	52.84±1.67^{a}	47.99±1.01^{b}	48.05±0.74^{b}	48.39±1.44^{b}	3.45*	0.02
IVDN as % of total N	39.78±2.11^{ab}	29.98±2.08^{c}	45.46±2.61^{a}	35.45±2.87^{bc}	7.22**	0.00
Methane gas production (%)	14.19±0.47^{c}	8.42±0.76^{a}	10.80±1.30^{ab}	12.33±0.65^{bc}	8.19**	0.00

Average of 12 values with S.E.

*Significant at 5% level (p<0.05) ** Significant at 1% level (p< 0.01)

Means bearing different superscripts within the same rows differ significantly

Table 6. Scorecard after assigning a grade to each parameter

Treatment	ME (MJ/kg DM)	DOM (%)	IVDN (as % of total N)	Methane gas production (%)	Total
T-1 (0)	2	2	0	5	9
T-2 (0.5%)	1	1	0	6	8
T-3 (0.25%)	1	1	4	6	12
T-4 (0.125%)	1	1	0	5	7

2015). Thus, the supplementation of aqueous extract of *P. guajava* leaves reduced the IVDN content at high concentrations. Hence 0.25 per cent of inclusion of each additive produced significantly (p<0.01) higher IVDN compared to other treatment groups.

Methane production

Beharka *et al*. (1991) reported that when diets were supplemented with *Aspergillus oryzae* fermentation extract, the fungal cultures diverted ruminal hydrogen flow away from methane into propionate and butyrate. Besides, Santra *et al.* (2012) reported that the tannin present in the guava leaves directly acted upon rumen methanogens and suppressed its activity. Thus, the combined effect of *A. oryzae* and *P. guajava* leaf extract significantly (p<0.01) reduced the methane production at 0.5 and 0.25 per cent in the present study.

Summary

Based on the findings, an optimum availability of *in vitro* degradable nitrogen (IVDN) for improved growth and a significant reduction in methanogenesis is obtained by the synergistic supplementation of *Aspergillus oryzae* culture and *Psidium guajava* leaves aqueous extract powder each at 0.25 per cent of TMR on DM basis. Therefore, the combination of *A. oryzae* culture and aqueous extract powder of *P. guajava* leaves each at 0.25 per cent of basal feed can be recommended for feeding crossbred calves for enhanced growth and environmental sustainability.

Conflicts of interest

There were no conflicts of interest reported by the author (s).

Acknowledgement

We are grateful to Kerala Veterinary and Animal Sciences University for providing the facilities needed for carrying out the research.

References

AOAC [Association of Official Analytical Chemists]. 2016. *Official Methods of Analysis* (20th Ed.). Association of Official Analytical Chemists, Washington, D. C. pp. 2-69.

Azzaz, H.H., Aziz, H.A., Farahat, E.S. and Murad, H.A. 2015. Impact of microbial feed supplements on the productive performance of lactating nubian goats. *Global Vet*. **14**(4): 567-575.

Beharka, A.A., Nagaraja, T.G. and Morrill, J.L. 1991. Performance and ruminal function development of young calves fed diets with *Aspergillus oryzae* fermentation extract. *J. Dairy Sci.* **74**: 4326-4336.

Bhatta, R., Saravanan, M., Baruah, L. and Sampath, K.T., 2012. Nutrient content, *in vitro* ruminal fermentation characteristics and methane reduction potential of tropical tannin-containing leaves. *J. Sci. Food Agric.* **92**: 2929-2935.

Economic review. 2017. *Agriculture and allied sectors. Chapter 2. State Planning Board,* Thiruvananthapuram, Kerala. India. pp. 27-39.

Hariadi, B.T. and Santoso, B., 2010. Evaluation of tropical plants containing tannin on *in vitro* methanogenesis and fermentation parameters using rumen fluid. *J. Sci. Food Agric.* **90**: 456-461.

Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.* **28**: 7-55.

Pal, K., Patra, A.K., Sahoo, A. and Kumawat, P.K. 2015. Evaluation of several tropical tree leaves for methane production potential, degradability and rumen fermentation *in vitro. Livest. Sci.* **180**: 98-105.

Patra, A.K. and Saxena, J. 2010. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. *Phytochemistry* **71**: 1198-1222.

Purushothaman, S., Shyama, K., Ally, K., Dildeep, V., Rani, K.J. and Sunanda, C. 2019. *In vitro* evaluation of tree fodders as an alternate feed source using–IVGPT. *J. Vet. Anim. Sci*, **50**: 95-99.

Raab, L., Cafantaris, B., Jilg, T. and Menke, K.H. 1983. Rumen protein degradation and biosynthesis: 1. A new method for determination of protein degradation in rumen fluid *in vitro*. *Br. J. Nutr.* **50**: 569-582.

Raseel, K. 2018. Evaluation of unconventional feed based complete rations for crossbred dairy cows in early lactation. *M.V.Sc. Thesis*, Kerala Veterinary and Animal Sciences University, Pookode, pp. 21-22.

Santra, A.S.A.B.K., Saikia, A. and Baruah, K.K. 2012. Scope of rumen manipulation using medicinal plants to mitigate methane production. *J. Pharmacogn.* **3**: 115-120.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods.* (8th Ed.). The Iowa State University Press, Ames, IA, USA. 314p.

Sosa, A., Galindo, J., Bocourt, R., Rodríguez, R., Albelo, N. and Oramas, A. 2010. Effect of *Aspergillus oryzae* on the rumen fermentation of *Pennisetum purpureum* cv. Cuba CT-115 through the *in vitro* gas technique. *Cuba. J. Agric. Sci.* **44**(2): 151-155.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **74**: 3583-3597.

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