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Use of faecal liquor: An alternate strategy for *in vitro* rumen fermentation studies in Pulikulam cattle, a native cattle breed of Tamil Nadu

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Abstract
Rumen liquor is used as a source of inocula for rumen *in vitro* fermentation and degradation studies. Fistulated animals are required to collect the rumen liquor. Maintaining fistulated animals raises ethical issues. Moreover, in field condition it is difficult to collect rumen liquor from Pulikulam cattle using rumen pump, owing to its ferocious nature. Hence, this study planned to compare Pulikulam cattle rumen liquor vs Pulikulam cattle faeces as a source of inocula for studying *in vitro* rumen fermentation characteristics of forages consumed by Pulikulam cattle in its breeding tract. The rumen liquor and faecal samples were obtained from three Pulikulam cattle reared in Pulikulam Cattle Research Station, Tamil Nadu, India. Rumen liquor was collected *via* rumen pump and fresh fecal samples were collected per rectum. Predominant forages present in the three districts, of Pulikulam cattle breeding tract were collected and their *in vitro* rumen fermentation characteristics for 24 and 48 hours were studied using either rumen liquor or faecal liquor as inocula. In all the three districts, no significant variation was observed in *in vitro* rumen fermentation characteristics of the predominant forages when either rumen liquor or faecal liquor from Pulikulam cattle were used as source of inocula. Hence, it was concluded that as an alternate strategy faecal liquor could be used as inoculam for *in vitro* rumen fermentation studies in Pulikulam cattle.

Keywords: Faecal liquor, forages, *in vitro* fermentation studies, Pulikulam cattle, rumen liquor

1. Introduction
Determining the nutritive value of forages present in grazing tracts of cattle, especially the indigenous breeds is very important to assess the nutrient intake by the animals and predict animal performance. *In vitro* rumen fermentation studies have been accepted worldwide as a reliable method to predict digestibility of feed stuffs. Conventionally rumen liquor is being used as inocula source for such studies. Rumen liquor may be collected from fistulated animals or from slaughtered animals (Cutrignelli *et al.*, 2005; Hughes *et al.*, 2012) [4, 6] or through oesophagus (Mould *et al.*, 2005) [10]. Maintaining fistulated animals without infection is difficult and it may create ethical issue related to surgical modification of animal. Collection from slaughtered animals gives a large amount of variability and when rumen pump is used for collection admixture with saliva poses a problem. In ferocious animals it is difficult to collect rumen liquor through using rumen pump.
Hence, several studies have been carried out to find alternate inocula for rumen fermentation studies. In recent year faecal liquor from ruminants have been investigated as an alternate inocula for *in vitro* fermentation studies (Mauricio *et al.*, 2001; Ramin *et al.*, 2015)[8, 12] Using fresh faeces as an alternate inocula has many advantages. It is easy to collect feces from the vigorous animals and no need to maintain fistulated animals. But it also has some limitation; one of the most important is lower enzymatic activity compared to rumen liquor (Mauricio *et al.*, 2001; Akhter *et al.*, 1999) [8, 1].
Pulikulam cattle are native breed of Tamil Nadu reared in Sivaganga, Madurai and Virudhunagar districts of the state.

Though these animals are comparatively smaller in size with the average body weight of around 200kg they are very active and ferocious animals (Srinivasan and Sathiamoorthy, 2020) [14].

The bulls of this breed are mainly maintained for bull baiting game or *Jallikkattu* which is one of the cultural sporting events conducted during harvesting season in Tamil Nadu, India. The nomadic rearers of Pulikulam cattle follow grazing practice only as feeding management (Srinivasan *et al*., 2021) [15]. This is a draught breed hence, animals are mainly maintained for manure. The *in vitro* rumen fermentation characteristics of forages present in the Pulikulam cattle breeding tract have not been studied. Hence, this study was conducted with the objective of evaluating *in vitro* rumen fermentation characteristics of forages of Pulikulam cattle breeding tract using rumen liquor and fecal liquor collected from the Pulikulam animals.

2. Materials and Methods

The study was conducted during the north east monsoon season at Pulikulam Cattle breeding tract. Forages were collected from Pulikulam cattle breeding tract *viz.*, Sivaganga, Madurai and Virudhunagar districts of Tamil Nadu. Based on the botanical composition predominant three forages were selected from each district.

Table 1: Composition (DMB) of Ration for Pulikulam cattle used in the collection of rumen liquor and faeces

S. No	Ingredient	Quantity (Kg)
1	Cumbu Napier-5 grass	2.25
2	Fodder sorghum-29	0.75
3	Concentrate feed	1.35
Concentrate Ingredient composition of concentrate feed		
S. No	**Ingredient**	**% Inclusion**
1	De oiled Rice bran	21.00
2	Sunflower oil cake	10.00
3	Bajra	5.00
4	Maize	18.00
5	Broken rice	10.00
6	Copra cake	15.00
7	Groundnut cake	5.00
8	Black gram Chunni	12.00
9	Mineral Mixture	0.10
10	Salt	1.00
11	Calcite	2.80
12	Sodium bicarbonate	0.09
13	Molasses aroma	0.01

The rumen liquor and faecal samples were obtained from the three Pulikulam cattle reared in Pulikulam Cattle Research Station. The ration presented in table 1 was used for feeding the animals utilized for collection of rumen liquor and faeces. Rumen fluid was collected from the three Pulikulam cattle using a pedel operated stomach tube (Bioplus®, Pedal suction apparatus BE-SU03, India). After discarding the initial volume, an additional 1000 ml of ruminal fluid was collected per animal. The collected ruminal fluids were mixed and strained through four-layer cheese cloth with continuous flushing of CO_2. Fresh fecal samples were collected from three Pulikulam cattle prior to morning feeding directly from the rectum and immediately placed in a pre-warmed thermos flask flushed with carbon dioxide and brought to laboratory maintaining strict anaerobic conditions during transit. The fecal liquor was prepared as per the procedure of Mauricio *et al.* (2001) [8].

The collected forages were suitably processed and subjected to the Hohenheim gas production technique as per the procedure of Menke *et al*., (1979) [9] and methane is estimated using saturated potassium hydroxide solution. *In vitro* degradation studies was determined as per Blummel *et al.* (1997) [2]. The *in vitro* rumen fermentation characteristics *viz.*, total gas, methane, *in vitro* apparent dry matter degradability, *in vitro* true dry matter degradability, microbial biomass, total volatile fatty acids, acetate, propionate and butyrate were assessed between two different inocula sources *viz.*, rumen liquor and faecal liquor. Thus, for each of the forage sample the study was carried out with two different inocula sources in triplicate, in two runs for 24 and 48 hrs. of incubation period.

Data were analyzed using IBM® SPSS® [16] Statistics version 20.0 for Windows® software as per the Snedecor and Cochran (1989) [13] for analysis of variance (ANOVA), linear regression analysis and independent sample T test. The critical difference between the groups was analyzed by Duncan's multiple range tests.

3. Results

3.1 Total gas production

In vitro total gas production from predominant forages collected in Pulikulam cattle breeding tract is presented in table 2. *Corchorus olitorius* gave the significantly (p<0.05) highest total gas production. No significant (p>0.05) variations were observed in total gas production between forages at 48 hours incubation, either when rumen liquor or faeces was used as source of inoculum. In Madurai district both at 24 and 48 hours of incubation no significant (p>0.05) variations were observed in total gas production. *Echinochloa colona* showed significantly (p<0.05) highest total gas production at both 24 and 48 hours, when rumen liquor was used as inoculum in Virudhunagar district. With regard to faecal liquor, no significant (p>0.05) variations were observed in total gas production, between forages in Virudhunagar district. The total gas production values for various forages, in all three districts, did not differ significantly (p>0.05) when either rumen liquor or faecal liquor, except with regard to *Perotis indica* from Madurai district, *Corchorus olitorius* gave the significantly (p<0.05) highest total gas production may be due to high available carbohydrate. Idris *et al.* (2009) [7] also reported high available carbohydrate in this forage. Similarly, *Echinochloa colona* showed significantly (p<0.05) highest total gas production at both 24 and 48 hours, when rumen liquor was used as inoculum in Virudhunagar district probably due to its high NFE content. Contrary to this study Cone *et al*. (1996) [3] concluded that cow rumen fluid cannot be replaced by cow faeces for determination of 24 hr. gas production, but it was found to be a good alternative for cow rumen fluid to accurately determine 48 hr. gas production.

Table 2: *In vitro* total gas production and methane (ml/0.2g substrate) from predominant forages collected in Pulikulam cattle breeding tract (Mean * ±SE)

S. No.	Botanical name	24 hours (N = 6)		48 hours (N = 6)		24 hours (N = 6)		48 hours (N = 6)	
		In vitro total gas production				*In vitro* methane production			
		Ruminal Liquor	Faecal Liquor	Ruminal Liquor	Faecal Liquor	Ruminal Liquor	Faecal Liquor	Ruminal Liquor	Faecal Liquor
Sivaganga District									
1.	*Corchorus olitorius*	9.25^{b}±0.62	7.75^{b}±1.25	13.25±2.17	13.50±2.39	1.05^{b}±0.15	1.45±0.15	2.15±0.28	3.02±0.92
2.	*Dactyloctenium aegyptium*	4.25^{a}±0.62	4.25^{a}±0.85	9.50±2.50	7.75±0.62	1.80^{a}±0.12	1.32±0.31	1.97±0.58	2.07±0.31
3.	*Pennisetum clandestinum*	4.25^{a}±0.85	5.25^{ab}±1.89	10.75±0.75	9.75±2.0	1.30^{b}±0.12	0.97±0.27	1.65±0.19	1.8±0.24
Madurai District									
1.	*Ocimum basilicum*	6.25±0.47	6.25±1.10	9.00±1.77	11.25±0.47	0.82±0.16	1.05±0.26	2.05±0.93	1.40±0.27
2.	*Pennisetum clandestinum*	7.00±1.41	6.00±0.57	11.25±1.10	12.00±1.87	1.35±0.21	1.55±0.25	3.25±1.06	2.47±0.36
3.	*Perotis indica*	8.75^{I}±0.25	6.25^{II}±0.47	10.50^{I}±0.64	14.00^{II}±0.70	1.22±0.22	1.97±0.40	2.15±0.15	2.15±0.41
Virudhunagar District									
1.	*Aristida setacea*	4.75^{ab}±0.75	5.00±0.577	10.75^{b}±1.43	12.00±3.58	2.42^{b}±0.45	2.05^{b}±0.27	2.40±0.40	3.52^{b}±0.49
2.	*Echinochloa colona*	6.00^{b}±0.40	5.75±1.03	14.00^{b}±1.08	10.75±2.86	1.27^{a}±0.35	1.32^{a}±0.11	2.00±0.39	2.90^{ab}±0.35
3.	*Pennisetum clandestinum*	3.50^{a}±0.64	3.50±0.28	6.00^{a}±0.40	6.50±0.50	1.25^{a}±0.09	1.65^{a}±0.19	1.27±0.22	1.72^{a}±0.39

Means bearing different alphabets as superscripts in the same column within the district differ significantly (p<0.05)
Means bearing different roman letter as superscripts in the same row within the respective incubation period within the district differ significantly (p<0.05)

Table 3: *In vitro* apparent dry matter degradability and *In vitro* true dry matter degradability (% DMB) of predominant forages collected in Pulikulam cattle breeding tract (Mean±SE)

S. No.	Botanical name	24 hours (N = 6)		48 hours (N = 6)		24 hours (N = 6)		48 hours (N = 6)	
		In vitro apparent dry matter degradability				*In vitro* true dry matter degradability			
		Ruminal Liquor	Faecal Liquor	Ruminal Liquor	Faecal Liquor	Ruminal Liquor	Faecal Liquor	Ruminal Liquor	Faecal Liquor
Sivaganga District									
1.	*Corchorus olitorius*	24.64±7.29	20.17^{a}±3.24	24.70±2.86	31.30±5.26	38.02±9.04	24.99^{a}±2.64	43.01±8.46	37.69±5.72
2.	*Dactyloctenium aegyptium*	26.54±5.03	32.40^{b}±2.97	26.93^{I}±2.34	33.48^{II}±3.87	34.22±5.72	39.39^{b}±3.53	38.37±4.77	41.00±4.57
3.	*Pennisetum clandestinum*	19.54^{I}±1.93	27.18^{abII}±1.53	22.33±2.26	29.85±3.32	32.26±5.68	38.14^{b}±3.96	36.63±4.02	38.92±2.41
Madurai District									
1.	*Ocimum basilicum*	14.07±2.44	22.75±5.33	15.42^{a}±2.01	23.31±4.04	26.78^{ab}±7.64	26.94^{ab}±4.46	32.92^{ab}±4.20	30.25±3.76
2.	*Pennisetum clandestinum*	14.23±4.41	20.16±3.09	21.66^{ab}±2.27	23.61±3.98	28.61^{a}±3.63	25.55^{a}±3.32	28.68^{a}±2.78	32.39±3.59
3.	*Perotis indica*	19.23±3.86	25.00±2.86	25.93^{b}±2.96	26.03±3.95	44.43^{b}±5.14	38.66^{b}±3.63	43.62^{b}±4.63	38.73±2.77
1.	*Aristida setacea*	23.76±7.55	24.77±3.13	30.88±3.85	26.35±2.46	27.00±7.69	29.79±2.41	37.25±4.14	33.18±3.67
2.	*Echinochloa colona*	11.17±3.65	16.42±3.49	26.44±6.21	23.55±4.36	16.37±3.76	24.04±3.91	39.09±7.50	29.95±4.48
3.	*Pennisetum clandestinum*	17.37±1.03	20.09±4.70	23.98±3.43	23.43±3.02	26.71±1.99	25.28±4.56	35.48±2.54	31.29±2.95

Means bearing different alphabets as superscripts in the same column within the district differ significantly (p<0.05)
Means bearing different roman letter as superscripts in the same row within the respective incubation period within the district differ significantly (p<0.05)

3.2 Methane Production

Table 2 presents observations of *in vitro* methane production from predominant forages collected in Pulikulam cattle breeding tract. In Sivaganga district, at 24 hours of incubation, when rumen liquor was used as source of inoculum significantly (p<0.05) lowest methane production was observed in *Dactyloctenium aegyptium.* However, at 48 hours of incubation when rumen liquor was used as source of inoculums and both in 24 and 48 hours of incubation when faecal liquor used as source of inoculum, methane production did not differ significantly (p>0.05).In Madurai district no significant (p>0.05) variations were observed in methane production, between forages when rumen liquor or faecal liquor were used as source of inoculums at both 24 and 48 hours of incubation. In Virudhunagar district, at 24 hours of incubation *Echinochloa colona and Pennisetum clandestinum* both had significantly (p<0.05) lower methane production when either rumen liquor or faecal liquor were used. The same trend was repeated at 48 hours of incubation, however, no significant (p>0.05) variation in methane production was observed among the forages at 48 hours incubation when rumen liquor was used as inoculums. Significantly lower methane production, were observed in *Dactyloctenium aegyptium* in Sivaganga district when rumen liquor was used as inoculum and *Echinochloa colona and Pennisetum clandestinum* in Virudhunagar district when both rumen liquor and feces were used as inoculum, indicating the potential of these forages for mitigating enteric methane emission.

3.3 *In vitro* apparent / true degradability and microbial biomass

In vitro apparent dry matter degradability (IVADMD) of predominant forages collected in Pulikulam cattle breeding tract is presented in table 3. In Sivaganga district, the IVADMD exhibited no significant (p>0.05) variation between forages at 24 hours incubation when rumen liquor was used as inocula also at 48 hours when both rumen liquor and faeces were used as inocula. At 24 hours of incubation using faecal inocula, IVADMD was significantly (p<0.05) highest in *Dactyloctenium aegyptium.* The source of inocula also significantly influenced IVADMD. In Madurai district, the IVADMD exhibited no significant (p>0.05) variation between forages at 24 hours incubation when rumen liquor or faecal liquor was used as inocula and also at 48 hours when feces was used as inocula. In Virudhunagar district, the IVADMD

exhibited no significant (p>0.05) variation between forages at 24 and 48 hours incubation in both inocula.

In vitro true dry matter degradability (IVADMD) of predominant forages collected in Pulikulam cattle breeding tract is presented in table 3. In Sivaganga district, the IVTDMD exhibited no significant (p>0.05) variation between forages at 24 hours incubation when rumen liquor was used as inocula and also at 48 hours when both rumen liquor and faecal liquor was used as inocula. At 24 hours of incubation using fecal inocula, IVTDMD was significantly (p<0.05) highest in *Dactyloctenium aegyptium.* In Madurai district IVTDMD was significantly (p<0.05) highest in *Perotis indica* at 24 hours incubation for both inoculum sources. In Virudhunagar district, the IVTDMD exhibited no significant (p>0.05) variation between forages at 24 and 48 hours incubation in both inocula sources. The results of the study concurred with Posada *et al*. (2012) [11] the authors had stated that when ruminal fluid and feces were compared for their ability to degrade dry matter in all substrates, it was found that only at 6, 12, and 24 hr., variations were present but not at the end of the fermentation process indicating that the extent of degradation was similar between both inocula. Mauricio *et al.* (2001) [8] concluded that, with forages of contrasting digestibility, faecal matter has potential as an alternative inoculum to rumen liquor for the *in vitro* gas production techniques; although with faeces based inoculum a consistently longer lag phase was observed.

Table 4: *In vitro* microbial biomass production (% DMB) from predominant forages collected in Pulikulam cattle breeding tract (Mean±SE)

S. No.	Botanical name	24 Hours (N = 8)		48 Hours (N = 4)	
		Ruminal Liquor	Faecal Liquor	Ruminal Liquor	Faecal Liquor
Sivaganga District					
1.	*Corchorus olitorius*	13.36±5.05	4.81^{a}±0.80	18.30±9.69	6.38±0.80
2.	*Dactyloctenium aegyptium*	7.67±1.76	6.98^{ab}±0.78	15.44^{I}±2.82	7.52^{II}±1.14
3.	*Pennisetum clandestinum*	12.72±6.43	10.95^{b}±2.45	14.29±4.61	9.06±1.96
Madurai District					
1.	*Ocimum basilicum*	12.71^{ab}±5.46	4.19^{a}±0.96	13.25±3.50	6.94±0.57
2.	*Pennisetum clandestinum*	8.37^{a}±1.85	7.38^{a}±0.39	11.26±4.30	8.77±1.41
3.	*Perotis indica*	25.20^{b}±5.14	13.65^{b}±2.69	17.69±5.06	12.70±2.89
Virudhunagar District					
1.	*Aristida setacea*	3.23±1.43	5.02±1.05	6.37±0.94	6.82±1.25
2.	*Echinochloa colona*	5.19±0.92	7.62±1.72	12.64±6.39	6.39±0.60
3.	*Pennisetum clandestinum*	8.33±1.00	7.19±0.58	11.50±1.80	7.85±1.23

Means bearing different alphabets as superscripts in the same column within the district differ significantly (p<0.05)

Means bearing different roman letter as superscripts in the same row within the respective incubation period within the district differ significantly (p<0.05)

In vitro microbial biomass production of predominant forages collected in Pulikulam cattle breeding tract is presented in table 4. In Sivaganga district, the microbial biomass production exhibited no significant (p>0.05) variation between forages at 48 hours of incubation in both the inocula. In Madurai district, *in vitro* microbial biomass production was significantly (p<0.05) highest in *Perotis indica* at 24 hours incubation for both inocula sources. In Virudhunagar district, the *in vitro* microbial biomass production exhibited no significant (p>0.05) variation between forages at 24 and 48 hours incubation. *In vitro* microbial biomass production differed between forages due to high IVADMD and IVTMD. Microbial protein production is largely dependent upon the availability of energy generated by the fermentation of carbohydrates, also the efficiency of MBP production varied widely between forages (Harun and Sali, 2019) [5].

3.4 Total volatile fatty acid (TVFA) production

In vitro total volatile fatty acids production from predominant forages collected from Pulikulam cattle breeding tract is presented in table 5. No significant variation (p>0.05) existed in the TVFA production at both 24 and 48 hours, of forages when the source of inocula varied except in the case of *Pennisetum clandestinum* at 48 hours of incubation where rumen liquor usage resulted in significantly (p<0.05) higher TVFA.

Table 5: *In vitro* total volatile fatty acids (m*M*) production from predominant forages collected in Pulikulam cattle breeding tract (Mean ± SE)

S. No.	Botanical name	24 hours (N = 6)		48 hours (N = 6)	
		Ruminal Liquor	Faecal Liquor	RUMINAL Liquor	Faecal Liquor
Sivaganga District					
1.	*Corchorus olitorius*	12.16^{b}±1.00	14.67±1.87	11.33^{a}±1.13	10.00±0.90
2	*Dactyloctenium aegyptium*	8.24^{a}±0.40	10.71±0.42	11.65^{a}±0.67	11.68±1.89
3	*Pennisetum clandestinum*	8.84^{a}±0.01	9.45±2.34	16.72^{bII}±0.60	10.33^{I}±0.25
Madurai District					
1.	*Ocimum basilicum*	17.18^{b}±1.52	19.86^{b}±0.83	17.43±3.27	13.65±1.71
2.	*Pennisetum clandestinum*	9.75^{a}±1.40	9.44^{a}±0.69	14.69±1.24	14.64±0.32
3.	*Perotis indica*	15.74^{bI}±0.11	10.19^{bII}±0.86	17.27^{I}±0.86	9.91^{II}±2.48
Virudhunagar District					
1.	*Aristida setacea*	10.68^{bI}±0.90	15.63^{aII}±1.19	17.56±4.55	11.04±0.70
2.	*Echinochloa colona*	26.80^{aI}±1.58	9.45^{bII}±0.68	14.96^{I}±1.62	8.74^{II}±0.29
3.	*Pennisetum clandestinum*	12.4^{b}±1.61	8.13^{b}±1.67	16.71±0.49	15.86±3.61

Means bearing different alphabets as superscripts in the same column within the district differ significantly (p<0.05)

Means bearing different roman letter as superscripts in the same row within the respective incubation period within the district differ significantly (p<0.05)

For forages in Madurai district at 48 hours of incubation, no significant (p>0.05) variation was observed in TVFA production between forages and inocula. In Virudhunagar district at 48 hours of incubation no significant (p>0.05) variation was observed in TVFA production across forages for both sources of inocula. In this district significant (p<0.05) variation in TVFA production when the source of inocula varied was in *Echinochloa colona* for both incubation periods and in *Aristida setacea* at 24 hours of incubation. Highest TVFA could be due to the higher NFE in these forages leading to better degradability of dry matter resulting in higher production of TVFA. The same reasoning can be applicable for *Ocimum basilicum*, which had high TVFA for both sources of inocula at 24 hours in Madurai district. Van Soest, (1994) [17] had stated that feed with higher non-structural carbohydrate would have higher production of volatile fatty acids.

4. Conclusion

Results from the current study concluded that as an alternate strategy faecal liquor could be used as alternate inocula source for *in vitro* rumen fermentation studies in Pulikulam cattle.

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