

Evaluation of Unconventional Feed Sources by In vitro Gas Production Technique Using Crossbred Cattle as Donors

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Abstract

A study was conducted to evaluate various unconventional feed ingredients by in vitro gas production technique (IVGPT). Six crossbred cattle of age 9-12 months were randomly selected from University Livestock Farm, Mannuthy. All the animals were maintained on total mixed ration (TMR) containing concentrate mixture Type II prepared using conventional feed ingredients and Hybrid Napier grass in 50:50. These animals were used as rumen liquor donors for conducting IVGPT. Thirty locally available unconventional feeds were collected from Thrissur, Kerala and their proximate analysis was done. Thirty iso-caloric and iso-nitrogenous total mixed rations containing 13- 14 per cent crude protein and 65-70 per cent total digestible nutrients were prepared with these unconventional feed ingredients replacing conventional ingredients on nutrient basis. These thirty TMR ' s were then subjected to IVGPT to estimate various fermentation parameters. The cluster analysis of the results revealed that among the unconventional feeds dhanwantharam thailam residues and rape seed cake can be utilized as ruminant livestock feed ingredients.

Keywords: Cluster Analysis, IVGPT, Parameters, Rumen Fermentation, Total Mixed Ration, Unconventional Feeds

Introduction

Optimum and balanced feeding has to be practiced for attaining maximum growth and production of livestock. The limited availability of nutritionally balanced feed and high cost incurred in feeding are the two major constraints for attaining these goals. The shrinking size of cultivable land is the limiting factor for availing quality fodder and increasing human population limits the availability of cereals, pulses and beans for feeding cattle (Jeong *et al.*, 2016). The cost of feeding has increased to about 80 per cent of the total cost of livestock rearing and there is a huge gap between feed and fodder requirement and availability (Economic Review, 2017). This tempted the scientists to tryout newer low cost but nutritionally rich feed resources as alternate feeds. The major share of these unconventional feeds is being contributed by agricultural by-products and residues, fruit and vegetable processing industry and ayurvedic pharmaceutical industry byproducts. Many ayurvedic pharmaceutical by-products, which are available in large quantities can be used as livestock feedstuffs, which otherwise are wasted and cause pollution. The unconventional fiber source includes various tree fodders which can contribute good quality and quantity protein and secondary metabolites with positive effects such as improved daily gain, voluntary feed intake, protective effect on protein utilization and decreasing parasitic load in animals (Peng *et al.*, 2016). Evaluation of the nutritive value of unconventional feed ingredients has to be done prior to incorporation in animal feeds to study the fermentation changes. *In vitro* gas production technique can be used as a tool for assessing rumen fermentation pattern (Mould *et al.*, 2005). The *in vitro* results will mimic the fermentation characteristics like substrate digestion, volatile fatty acid production, nutrient digestibility and microbial protein production as *in vivo* conditions (Kaiser and Weniger, 1994). This method will be helpful when large numbers of samples are to be evaluated. The study was conducted to evaluate fermentation of total mixed ration (TMR) containing various unconventional feed ingredients by *in vitro* gas production technique using crossbred cattle as donor animals and to select two best TMR containing unconventional ingredients.

Materials and Methods

Donor Animals

Six crossbred female calves of 9-12 months age were selected from University Livestock Farm, Mannuthy, Kerala, India. These animals were maintained under uniform management conditions. Prior to the study all the animals were dewormed using albendazole suspension for controlling endoparasites. All the animals were fed with TMR containing concentrate mixture type II (BIS-2012) prepared using conventional feed ingredients (Table 1) and hybrid Napier grass in 50:50.

Table 1: Ingredient composition of control concentrate mixture fed to donor animals

Ingredient	Quantity (parts per quintal), %
Maize	31.5
Bajra	9.5
Soya bean meal	22
Wheat bran	9.5
Deoiled rice bran	23
Mineral mixture	3
Salt	1.5
Total	100

Unconventional Feeds

Thirty unconventional feeds such as cooked barley (*Hordeum vulgare*), dhanwantharam thailam residue, spent grapes (*Vitis vinifera*), maize waste (*Zea mays*), tapioca waste (*Manihot esculenta*), jack fruit seeds (*Artocarpus heterophyllus*), mango kernel (*Mangifera indica*), spent rosemary (*Rosmarinus officinalis*), soya sauce waste (*Glycine max*), banana peels (*Musa paradisiaca*), ksheerabala residues, spent cumin (*Cuminum cyminum*), turmeric waste (*Curcuma longa*), elephant palm leaves (*Caryota urens*), rain tree pods (*Albizia saman*), azolla (*Azolla filiculoides*), rapeseed cake (*Brassica napus*), desmanthus (*Desmanthus virgatus*), gliricidia leaves (*Gliricidia sepium*), moringa leaves (*Moringa oleifera*), coconut leaves (*Cocos nucifera*), banana leaves (*Musa paradisiaca*), Areca sheath (*Areca catechu*), oil palm leaves (*Elaies guineensis*), caliandra (*Caliandra haematocephala*), Jack

leaves (*Artocarpus heterophyllus*), Venga leaves (*Pterocarpus marsupium*), spent coconut gratings, sugarcane baggase (*Saccharum officinarum*) and agathi (*Sesbania grandiflora*) were collected from different locations of Thrissur, Kerala and their proximate analysis was done (AOAC,2012). These ingredients were then grouped into protein, energy and fiber sources. Thirty iso caloric and iso nitrogenous total mixed rations containing 13- 14 per cent crude protein and 65-70 per cent total digestible nutrient were prepared by using these unconventional feed ingredients replacing conventional ingredients from control concentrate mixture and green grass in 50:50 and presented in (Table 2). The proximate analysis of all the thirty TMRs was done as per standard procedure (AOAC, 2012) (Table 3).

***In Vitro* Gas Production Technique (IVGPT)**

The thirty TMRs were subjected to *in vitro* trials according to the procedure described by Menke and Steingass (1988). Rumen liquor was collected from the crossbred calves 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 IVGPT.

Total Gas Production

Gas produced (ml/200mg substrate) by fermentation of substrate feed during 24 hour was measured after correcting corresponding blank values (Menke and Steingass, 1998).

***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-

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

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

Microbial Biomass Production (MBP)

Microbial biomass production (MBP) of the TMR tested was calculated from TDOM using equation, MBP (mg) = TDOM (mg) – (Corrected gas production for 24 hrs×2.20)

Where 2.20 is the stoichiometric factor for mixed diets (Blummel and Lebzien, 2001).

Metabolizable Energy (ME)

ME of target TMR was calculated by the method of (Menke and Stienass, 1988).

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

Where, CP - Crude protein percentage, EE - Ether extract percentage, GP- corrected gas production for 24 hours.

Estimation of Volatile Fatty Acids

Volatile fatty acid composition of the inoculum was found out using 7890A GC System gas chromatograph, (Agilent Technologies, USA) as per standard procedure described by Filípek and Dvorak (2009). On completion of the incubation, the buffered rumen liquor was filtered through four layers of muslin cloth and approximately 0.8 ml of the sample was preserved with 200 µl of 25% HPO₃. The samples preserved were immediately analyzed or stored at -20°C temperature for later analysis.

Methane Estimation

Methane production capacity of the TMR was determined by using methane sensor fabricated analyzer developed in Kerala Veterinary and Animal Sciences University.

Cluster Analysis

Hierarchical cluster analysis was done for grouping 30 TMR into different clusters using the parameters TDMD (%), TOMD (%), METHANE (%), ME (KJ/KgDM), MBP (mg) and TVFA (mmol/L). Algorithm used for grouping is the average linkage between groups. Based on that a dendrogram was prepared. Allowing 30 per cent variability within group five clusters were identified. Two total mixed rations were selected from the best cluster based on observations such as improved organic matter degradability, total volatile fatty acids, improved microbial biomass production, high metabolizable energy and low methane production. Data gathered on the various parameters were analyzed statistically as per Snedecor and Cochran (1994) using the software, statistical programme for social sciences (SPSS) version 24.0.

Table 2: Ingredient composition of 30 concentrates mixture for preparation of TMR

TMR-1

Ingredient	Quantity (parts per quintal),%
Maize	29
Bajra	7
Spent cumin	6
Soya bean meal	21
Wheat bran	10.5
De oiled rice bran	22
Mineral mixture	3
Salt	1.5
Total	100

TMR-2

Ingredient	Quantity (parts per quintal), %
Maize	29
Bajra	7
Dried azolla	5
Soya bean meal	21
Wheat bran	11.5
De oiled rice bran	22
Mineral mixture	3
Salt	1.5
Total	100

TMR-3

Ingredient	Quantity (parts per quintal),%
Maize	29
Bajra	9
Rape seed cake	5
Soya bean meal	18
Wheat bran	20.5
De oiled rice bran	14
Mineral mixture	3
Salt	1.5
Total	100

TMR-4

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-5

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

TMR-6

Ingredient	Quantity (parts per quintal), %
Maize	30
Bajra	9
Dried 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-7

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

TMR-8

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

TMR-9

Ingredient	Quantity (parts per quintal), %
Maize	30
Bajra	10
Dried sugarcane baggase	4
Soya bean meal	23
Wheat bran	15
De oiled rice bran	13.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-10

Ingredient	Quantity (parts per quintal), %
Maize	32
Bajra	2
Tapioca starch waste	5
Soya bean meal	24
De oiled rice bran	32.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-11

Ingredient	Quantity (parts per quintal), %
Maize	29
Bajra	7.5
Maize waste	5
Wheat bran	22
Soya bean meal	24
De oiled rice bran	8
Mineral mixture	3
Salt	1.5
Total	100

TMR-12

Ingredient	Quantity (parts per quintal), %
Maize	29
Bajra	5
Jack fruit seeds	5
Soya bean meal	22
Wheat bran	15.5
De oiled rice bran	19
Mineral mixture	3
Salt	1.5
Total	100

TMR-13

Ingredient	Quantity (parts per quintal),%
Maize	29
Bajra	5
Mango seed kernel	6
Soya bean meal	22
Wheat bran	10
De oiled rice bran	24.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-14

Ingredient	Quantity (parts per quintal),%
Maize	28
Bajra	5
Rain tree pods	7
Soya bean meal	22
Wheat bran	11.5
De oiled rice bran	22
Mineral mixture	3
Salt	1.5
Total	100

TMR-15

Ingredient	Quantity (parts per quintal),%
Maize	29
Bajra	7
Soya sauce waste	6
Soya bean meal	20
Wheat bran	9
De oiled rice bran	24.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-16

Ingredient	Quantity (parts per quintal),%
Maize	28
Bajra	7
Turmeric waste	5
Soya bean meal	22
Wheat bran	11.5
De oiled rice bran	22
Mineral mixture	3
Salt	1.5
Total	100

TMR-17

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

TMR-18

Ingredient	Quantity (parts per quintal),%
Maize	30
Bajra	8
Dried agathi	5
Soya bean meal	20
Wheat bran	19
De oiled rice bran	13.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-19

Ingredient	Quantity (parts per quintal),%
Maize	30
Bajra	5
Dried cooked barley	5
Soya bean meal	22
Wheat bran	20
De oiled rice bran	13.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-20

Ingredient	Quantity (parts per quintal), %
Maize	30
Bajra	5
Dried spent rosemary	5
Soya bean meal	22
Wheat bran	20
De oiled rice bran	13.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-21

Ingredient	Quantity (parts per quintal), %
Maize	30
Bajra	5
Dried ksheerabala residue	5
Soya bean meal	20
Wheat bran	21
De oiled rice bran	14.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-22

Ingredient	Quantity (parts per quintal), %
Maize	30
Bajra	6
Dried coconut leaves	6
Soya bean meal	23
Wheat bran	16
De oiled rice bran	14.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-23

Ingredient	Quantity (parts per quintal), %
Maize	30
Bajra	8
Dried oil palm leaves	5
Soya bean meal	23
Wheat bran	15
De oiled rice bran	14.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-24

Ingredient	Quantity (parts per quintal), %
Maize	30
Bajra	8
Dried caliandra leaves	5
Soya bean meal	21
Wheat bran	17
De oiled rice bran	14.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-25

Ingredient	Quantity (parts per quintal), %
Maize	31
Bajra	8
Dried vengra leaves	5
Soya bean meal	21
Wheat bran	16
De oiled rice bran	14.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-26

Ingredient	Quantity (parts per quintal), %
Maize	31
Bajra	6
Dried elephant palm leaves	5
Soya bean meal	23
Wheat bran	16
De oiled rice bran	14.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-27

Ingredient	Quantity (parts per quintal), %
Maize	30
Bajra	5
Dried dhanwantharam residues	5
Soya bean meal	23
Wheat bran	17
De oiled rice bran	15.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-28

Ingredient	Quantity (parts per quintal),%
Maize	30
Bajra	5
Dried spent grapes	5
Soya bean meal	23
Wheat bran	17
De oiled rice bran	15.5
Mineral mixture	3
Salt	1.5
Total	100

TMR-29

Ingredient	Quantity (parts per quintal),%
Maize	29
Bajra	7
Dried spent coconut gratings	6
Soya bean meal	21
Wheat bran	10.5
De oiled rice bran	22
Mineral mixture	3
Salt	1.5
Total	100

TMR-30

Ingredient	Quantity (parts per quintal),%
Maize	30
Bajra	7
Dried banana peals	5
Soya bean meal	23
Wheat bran	15
De oiled rice bran	15.5
Mineral mixture	3
Salt	1.5
Total	100

Results and Discussion

Thirty locally available unconventional feeds were analyzed for their chemical composition and presented in (Table 3). The proximate analysis revealed that protein rich feed resources such as soya sauce waste, spent cumin, azolla, rape seed cake, desmanthus, gliricidia leaves, moringa leaves, caliantra, ksheerabala residue and agathi were having a crude protein content ranging from 17.29 to 34.42 per cent and were used to replace the protein ingredients from concentrate. Energy rich materials such as tapioca starch waste, jack fruit seeds, mango seed kernel, turmeric waste, rain tree pods, spent coconut gratings, cooked barley, dhanwantharam thailam residues, maize waste and spent grapes were having high NFE content ranging from 37.73 to 84.00 per cent.

Table 3: Chemical composition of unconventional feeds, % DM basis

Coding	Un conventional feed	Moisture	Crude protein	Ether extract	Crude fiber	Total ash	Nitrogen free extract
1	Spent cumin	6.85	17.29	8.31	14.94	4.6	54.86
2	Azolla	95.56	21.71	3.91	14.94	1.91	57.53
3	Rape seed cake	9.13	34.42	3.23	13.3	7.64	41.41
4	Desmanthus	71.7	22.66	2.29	22.5	4.37	48.18
5	Gliricidia leaves	49.58	21.68	11.46	24.14	10	32.72
6	Moringa leaves	77.5	17.8	5.1	9.7	10.6	56.8
7	Banana leaves	76.1	10	5.6	23.1	13.3	48
8	Jack leaves	59.75	16.54	1.63	18.37	9.09	54.37
9	Sugarcane baggase	47.69	2.65	1.35	41.7	5.29	49.01
10	Tapioca starch waste	52.23	2	0.3	5.2	8.4	84
11	Maize waste	46.62	5.51	6.04	35.92	1.82	50.71
12	Jack fruit seeds	64.3	11.02	1.01	2.36	3.97	81.64
13	Mango seed kernel	44.4	7.6	13.5	1.5	2.8	74.6
14	Rain tree pods	17.12	15.31	1.5	10.07	3.19	69.93
15	Soya sauce waste	8.2	17.89	17.19	11.35	26.66	26.91
16	Turmeric waste	7.22	13.14	12.82	7.01	12	55.03
17	Areca sheath	11.9	3.5	0.07	59.5	6.4	30.53
18	Agathi	70.56	27.6	5.1	9.7	7.1	50.5
19	Cooked barley	13.55	9.23	3.43	6.28	2.14	78.92
20	Spent rosemary	7.79	7.1	0.41	6.4	6.72	69.31
21	Ksheerabala residues	7.5	29.52	13.26	6.39	8.42	59
22	Coconut leaves	60.35	10.57	4.49	17.2	1.07	31.7
23	Oil palm leaves	55.64	7.7	3.2	27.2	8.47	53.43
24	Caliandra	52	18.45	3.12	19.57	4	54.86
25	Venga leaves	59.75	14.9	4.2	26.3	10.5	44.1
26	Elephant palm leaves	61.54	7.7	3.4	31	9.6	48.3
27	Dhanwantharam thailam residues	15.48	11.99	17.7	25.2	5.89	39.22
28	Spent grapes	9.745	6.35	2.54	8.53	3.1	79.48
29	Spent Coconut gratings	32.39	3.02	20.46	28.14	2.78	45.6
30	Banana peels	86.57	8	1.7	31.7	8.5	50.1

Fiber sources such as banana, peels, elephant palm leaves, coconut leaves, banana leaves, areca sheath, oil palm leaves, jack leaves, vengal leaves, sugarcane baggase and spent rosemary contains crude fiber ranging from 17.2 to 59.5 per cent. Spent coconut gratings, dhanwantharam thailam residues and soya sauce waste were containing ether extract content ranging from 17.7 to 20.46 per cent. The TMR prepared using unconventional feed ingredients replacing conventional ingredients were analyzed for chemical composition and presented in (Table 4).

Table 4: Chemical Composition*, % DM basis of TMRs used in IVGPT

TMR	Dry* matter	Crude *protein	Ether* extract	Crude* fiber	NDF*	ADF*	Total* Ash	AIA*	NFE*	OM*
1	94.3	15.65	1.4	18.95	38.08	21.08	10.91	3.9	53.09	89.09
2	93.59	15.66	1.2	19.01	37	21.87	11.12	3.8	53.01	88.88
3	93.5	15.2	1.8	17.6	44.28	27.5	9.52	3.9	55.88	90.48
4	94.07	15.79	1.45	18.5	38.89	23.26	10.7	3.95	53.36	89.3
5	93.28	15.82	1.47	18.84	42.04	24.11	10.69	4	53.18	89.31
6	93.39	15.18	1.5	19	47.58	25.49	11.19	3.65	53.13	88.81
7	93.74	15.17	1.39	18.5	40.39	23.63	10.94	3.7	54	89.06
8	93.87	15.79	1.42	18.65	42.14	25.35	11	3.38	53.14	89
9	94.05	15.02	1.2	19.48	43.98	26.18	11.38	3.64	52.92	88.62
10	92.32	14.7	2	19.56	51.31	30.15	9.38	3.95	54.36	90.62
11	89.97	14.89	1.15	19.52	46.71	29.8	12.73	4.1	51.71	87.23
12	89.91	14.53	1.05	17.82	35.78	22.5	12.86	3.89	53.74	87.14
13	90.07	14.46	1.15	17.5	34.88	23.4	12.67	4	54.22	87.33
14	90.64	14.48	1.02	17.6	41.86	30.65	12.39	3.94	54.51	87.61
15	90.3	15.2	5.9	16.2	27.3	27.3	9.3	4.2	53.4	90.7
16	90.18	14.25	1.25	17.2	42.93	30.15	9.4	4	57.9	90.6
17	89.92	14.46	1.2	19.1	37.27	24.53	12.39	4.12	52.85	87.61
18	90.17	14.45	1.05	18.2	42.33	27.2	12.74	3.89	53.56	87.26
19	90.38	14.72	1	19.34	39.1	28.3	12.81	3.65	52.13	87.19
20	87.99	14.48	1.02	18.65	38.4	22.83	13.11	3.56	52.74	86.69
21	87.76	14.17	1.25	17.45	37.19	26.34	10.29	4.02	56.84	89.71
22	86.3	14.01	1.23	20.34	38.86	23.45	10.21	3.95	54.21	89.79
23	88.12	15.14	1.31	19.2	42.71	30.18	10.21	4.21	54.14	89.79
24	88.71	15.37	1.28	19.86	39.62	28.12	9.78	3.88	53.71	90.22
25	93.51	14.81	1.15	18.75	42.7	26.16	10.31	3.65	54.98	89.69
26	92.9	14.87	1.24	19.25	39.62	22.3	10.45	3.87	54.19	89.55
27	93.07	14.65	1.05	17.6	50.5	28	9.52	3.9	57.18	90.48
28	93.34	15.17	1.18	18.1	44.28	25.78	10.29	3.95	55.26	89.71
29	93.37	14.46	1.05	17.65	35.92	22.31	10.12	4	56.72	89.88
30	92.69	15.75	1.2	18.65	34.73	21.55	10.27	4.51	54.13	89.73

* Average of six values

Seo *et al.* (2015) evaluated spent coffee grounds (SCG) and *Artemisia princeps* as alternative to conventional feed ingredients. Similarly, Kaur and Thakur (2017) evaluated unconventional energy resources such as phalaris minor seeds and TMRs for proximate principles and recommended that these ingredients could be used as alternate feed sources for replacing conventional feed ingredients. The gas production for 24 hours was assessed and found to be ranging from 13 ± 0.58 to 21.8 ± 0.12 (ml/200 mg). The corresponding TDMD% ranged from 64.5 ± 0.29 to 80 ± 0.29 , while TOMD% ranged from 62.45 ± 0.58 to 80.68 ± 0.38 , methane % varied from 6.35 ± 0.03 to 13.5 ± 0.14 , MBP(mg/200 mg) ranged from 21.28 ± 0.01 to 49.62 ± 0.01 , ME ranged from 4.45 ± 0.03 to 5.55 ± 0.03 and TVFA ranged from 50.69 ± 0.06 to 94.78 ± 0.03 respectively. Chumpawade and Pimpa (2009) conducted studies on effect of leaves as fiber sources in total mixed ration on *in vitro* fermentation using IVGPT and concluded that highest rate of gas production, IVDMD, TOMD were observed in the TMR containing Siamese rough bush leaves as fiber source. Pang *et al.* (2014) studied the effect of *Enterococcus faecium* on *in vitro* ruminal fermentation rate and extend of three TMR named mixed forage ration with forage source as corn stakes using rumen liquor collected from Holstein dairy cows and it was reported that gas production ranged from 182 to 196 (ml/g DM) and TVFA

(μ) ranged from 132.4 to 137.2 μ).

Table 5: In vitro* evaluation of TMR in crossbred cows using IVGPT

TMR	Gas Production (ml/200mg)	TDMD%	TOMD %	Methane %	MBP (mg)	ME (MJ/kg DM)	TVFA (mmol/L)
1	13.00 ± 0.58	71.50 ± 0.29	70.18 ± 0.01	07.04 ± 0.02	42.91 ± 1.37	04.55 ± 0.03	80.77 ± 0.01
2	15.00 ± 0.29	72.00 ± 0.29	70.66 ± 0.02	07.92 ± 0.06	37.66 ± 0.01	04.83 ± 0.01	70.05 ± 0.03
3	14.50 ± 0.12	80.00 ± 0.29	80.68 ± 0.38	09.48 ± 0.12	48.83 ± 0.01	04.78 ± 0.01	94.78 ± 0.03
4	18.00 ± 0.29	69.50 ± 0.29	71.61 ± 0.73	08.60 ± 0.03	32.26 ± 0.01	5.32 ± 0.02	65.13 ± 0.09
5	15.00 ± 0.58	78.00 ± 0.29	77.41 ± 0.12	08.90 ± 0.03	44.41 ± 0.01	4.78 ± 0.01	68.51 ± 0.06
6	15.00 ± 0.29	71.50 ± 0.12	70.99 ± 0.23	08.50 ± 0.01	37.99 ± 0.01	04.78 ± 0.01	76.50 ± 0.03
7	15.00 ± 0.58	71.50 ± 0.29	71.81 ± 0.12	13.50 ± 0.14	38.81 ± 0.01	4.86 ± 0.01	50.69 ± 0.06
8	20.00 ± 0.58	72.50 ± 0.29	70.09 ± 0.60	07.72 ± 0.01	25.59 ± 0.01	5.50 ± 0.01	69.65 ± 0.03
9	17.00 ± 0.12	74.00 ± 0.29	71.51 ± 0.15	08.90 ± 0.02	34.14 ± 0.02	05.08 ± 0.02	74.05 ± 0.02
10	15.50 ± 0.29	78.50 ± 0.29	77.52 ± 0.12	7.53 ± 0.01	43.42 ± 0.01	05.08 ± 0.01	90.40 ± 0.12
11	19.00 ± 0.58	72.50 ± 0.29	68.97 ± 0.06	09.29 ± 0.06	27.17 ± 0.02	05.24 ± 0.01	80.30 ± 0.06
12	18.00 ± 0.23	70.00 ± 0.58	68.95 ± 0.58	08.70 ± 0.01	29.35 ± 0.03	05.11 ± 0.03	81.49 ± 0.01
13	18.00 ± 0.15	71.50 ± 0.29	71.13 ± 0.58	08.99 ± 0.01	31.60 ± 0.03	05.10 ± 0.03	50.69 ± 0.01
14	18.80 ± 0.12	70.00 ± 0.29	69.35 ± 0.33	06.74 ± 0.01	27.61 ± 0.01	05.22 ± 0.01	71.57 ± 0.01
15	18.50 ± 0.29	77.50 ± 0.29	77.68 ± 0.38	08.31 ± 0.03	37.01 ± 0.01	05.18 ± 0.01	95.14 ± 0.02
16	14.50 ± 0.29	79.50 ± 0.29	78.66 ± 0.33	09.09 ± 0.05	47.08 ± 0.01	04.56 ± 0.01	96.5 ± 0.06
17	19.00 ± 0.58	64.50 ± 0.29	62.45 ± 0.58	09.19 ± 0.06	21.28 ± 0.01	05.24 ± 0.01	72.65 ± 0.03
18	19.50 ± 0.29	73.00 ± 0.29	70.41 ± 0.58	08.2 ± 0.02	27.51 ± 0.01	05.3 ± 0.01	75.03 ± 0.02
19	21.80 ± 0.12	71.00 ± 0.29	69.45 ± 0.25	8.80 ± 0.03	21.52 ± 0.01	05.55 ± 0.03	77.50 ± 0.03
20	13.50 ± 0.29	75.50 ± 0.29	72.36 ± 0.20	08.80 ± 0.01	42.69 ± 0.01	04.45 ± 0.03	63.31 ± 0.02
21	14.10 ± 0.06	79.00 ± 0.29	80.74 ± 0.25	08.50 ± 0.01	49.62 ± 0.01	04.50 ± 0.01	92.70 ± 0.01
22	14.50 ± 0.29	77.00 ± 0.29	75.52 ± 0.12	07.62 ± 0.01	43.62 ± 0.02	04.52 ± 0.01	70.56 ± 0.02
23	14.00 ± 0.58	77.00 ± 0.12	75.49 ± 0.31	07.62 ± 0.02	44.49 ± 0.03	04.61 ± 0.03	65.40 ± 0.03
24	14.50 ± 0.29	72.00 ± 0.29	76.36 ± 0.21	07.53 ± 0.06	44.44 ± 0.02	04.61 ± 0.01	68.04 ± 0.02
25	14.50 ± 0.29	72.00 ± 0.29	76.36 ± 0.21	07.53 ± 0.06	35.15 ± 0.03	04.71 ± 0.02	77.53 ± 0.02
26	15.10 ± 0.06	78.50 ± 0.29	78.54 ± 0.29	07.82 ± 0.01	45.28 ± 0.01	04.74 ± 0.02	68.51 ± 0.02
27	14.10 ± 0.06	79.50 ± 0.29	78.64 ± 0.06	07.92 ± 0.01	48.60 ± 0.01	04.62 ± 0.02	85.74 ± 0.02
28	19.50 ± 0.29	76.00 ± 0.58	73.61 ± 0.58	07.92 ± 0.02	30.70 ± 0.03	05.42 ± 0.01	72.47 ± 0.01
29	18.00 ± 0.58	73.50 ± 0.29	72.84 ± 0.06	06.35 ± 0.03	33.34 ± 0.02	05.1 ± 0.03	64.97 ± 0.02
30	17.00 ± 0.58	72.00 ± 3.25	73.08 ± 0.01	07.33 ± 0.01	35.68 ± 0.01	05.14 ± 0.02	70.98 ± 0.01

*Mean values are based on three replicates

Elghandour *et al.* (2015) conducted in vitro evaluation of five TMR with different maize silage: concentrate ratio using rumen inoculum from two brown Swiss cows and they reported that gas production (ml/g DM) ranged from 120.6 to 202.7 ml/g DM at 24 hours incubation and they also reported dry matter digestibility that ranged from 68.9 to 88.01 per cent, ME of 5.7 to 8.7 MJ/kg DM, organic matter digestibility of 39.0 to 59.6 per cent. Seo *et al.* (2015) conducted in vitro evaluation of spent coffee grounds (SCG) and *Artimisia princeps* (APR) and evaluated gas production, methane emission and total volatile fatty acid estimation using rumen fluid from Holstein cows and obtained 184.6 ml/g DM and 180.7 ml/g DM, methane emission (ml/g DM) as 5.87 and 5.97 respectively for SCG and APR and TVFA production mmol/g DM 5.87 and 5.97 for SCG and APR, respectively.

Table 6: Cluster analysis of TMR in crossbred cows

TMR	TDMD %	TOMD %	Methane %	ME (MJ/kg DM)	MBP (mg)	TVFA (mmol/L)	Member ship
1	71.5 ± 0.29	70.18 ± 0.01	7.04 ± 0.02	4.55 ± 0.03	42.91 ± 1.37	80.77 ± 0.01	1
2	72 ± 0.29	70.66 ± 0.02	7.92 ± 0.06	4.83 ± 0.01	37.66 ± 0.01	70.05 ± 0.03	1
3	80 ± 0.29	80.68 ± 0.38	9.48 ± 0.12	4.78 ± 0.01	48.83 ± 0.01	94.78 ± 0.03	2
4	69.5 ± 0.29	71.61 ± 0.73	8.6 ± 0.03	5.32 ± 0.02	32.26 ± 0.01	65.13 ± 0.09	3
5	78 ± 0.29	77.41 ± 0.12	8.9 ± 0.03	4.78 ± 0.01	44.41 ± 0.01	68.51 ± 0.06	3
6	71.5 ± 0.12	70.99 ± 0.23	8.5 ± 0.01	4.78 ± 0.01	37.99 ± 0.01	76.5 ± 0.03	1
7	71.5 ± 0.29	71.81 ± 0.12	13.5 ± 0.14	4.86 ± 0.01	38.81 ± 0.01	50.69 ± 0.06	4
8	72.5 ± 0.29	70.09 ± 0.6	7.72 ± 0.01	5.5 ± 0.01	25.59 ± 0.01	69.65 ± 0.03	5
9	74 ± 0.29	71.51 ± 0.15	8.9 ± 0.02	5.08 ± 0.02	34.14 ± 0.02	74.05 ± 0.02	1
10	78.5 ± 0.29	77.52 ± 0.12	7.53 ± 0.01	5.08 ± 0.01	43.42 ± 0.01	90.4 ± 0.12	2
11	72.5 ± 0.29	68.97 ± 0.06	9.29 ± 0.06	5.24 ± 0.01	27.17 ± 0.02	80.3 ± 0.06	
12	70 ± 0.58	68.95 ± 0.58	8.7 ± 0.01	5.11 ± 0.03	29.35 ± 0.03	81.49 ± 0.01	1
13	71.5 ± 0.29	71.13 ± 0.58	8.99 ± 0.01	5.1 ± 0.03	31.6 ± 0.03	50.69 ± 0.01	4
14	70 ± 0.29	69.35 ± 0.33	6.74 ± 0.01	5.22 ± 0.01	27.61 ± 0.01	71.57 ± 0.01	5
15	77.5 ± 0.29	77.68 ± 0.38	8.31 ± 0.03	5.18 ± 0.01	37.01 ± 0.01	85.14 ± 0.02	2
16	79.5 ± 0.29	78.66 ± 0.33	9.09 ± 0.05	4.56 ± 0.01	47.08 ± 0.01	96.5 ± 0.06	1
17	64.5 ± 0.29	62.45 ± 0.58	9.19 ± 0.06	5.24 ± 0.01	21.28 ± 0.01	72.65 ± 0.03	5
18	73 ± 0.29	70.41 ± 0.58	8.2 ± 0.02	5.3 ± 0.01	27.51 ± 0.01	75.03 ± 0.02	5
19	71 ± 0.29	69.45 ± 0.25	8.8 ± 0.03	5.55 ± 0.03	21.52 ± 0.01	77.5 ± 0.03	1
20	75.5 ± 0.29	72.36 ± 0.2	8.8 ± 0.01	4.45 ± 0.03	42.69 ± 0.01	63.31 ± 0.02	3
21	79 ± 0.29	80.74 ± 0.25	8.5 ± 0.01	4.5 ± 0.01	49.62 ± 0.01	92.7 ± 0.01	4
22	77 ± 0.29	75.52 ± 0.12	7.62 ± 0.01	4.52 ± 0.01	43.62 ± 0.02	70.56 ± 0.02	1
23	77 ± 0.12	75.49 ± 0.31	7.62 ± 0.02	4.61 ± 0.03	44.49 ± 0.03	65.4 ± 0.03	3
24	72 ± 0.29	76.36 ± 0.21	7.53 ± 0.06	4.61 ± 0.01	44.44 ± 0.02	68.04 ± 0.02	1
25	72 ± 0.29	76.36 ± 0.21	7.53 ± 0.06	4.71 ± 0.02	35.15 ± 0.03	77.53 ± 0.02	1
26	78.5 ± 0.29	78.54 ± 0.29	7.82 ± 0.01	4.74 ± 0.02	45.28 ± 0.01	68.51 ± 0.02	5
27	79.5 ± 0.29	78.64 ± 0.06	7.92 ± 0.01	4.62 ± 0.02	48.6 ± 0.01	95.74 ± 0.02	2
28	76 ± 0.58	73.61 ± 0.58	7.92 ± 0.02	5.42 ± 0.01	30.7 ± 0.03	72.47 ± 0.01	1
29	73.5 ± 0.29	72.84 ± 0.06	6.35 ± 0.03	5.1 ± 0.03	33.34 ± 0.02	64.97 ± 0.02	3
30	17 ± 0.58	72 ± 3.25	73.08 ± 0.01	5.14 ± 0.02	35.68 ± 0.01	5.14 ± 0.02	5

Table 7: Average values of fermentation parameters in clusters

Member-ship	TDMD %	TOMD %	Methane %	ME (MJ/kgDM)	MBP (mg)	TVFA (MMOL/L)
1	72.07	70.68	7.98	5.12	31.91	73.87
2	79	78.99	8.47	4.79	45.76	92.54
3	76.33	75.95	8.05	4.62	44.16	67.39
4	71.5	71.47	11.25	4.98	35.21	50.69
5	64.5	62.45	9.19	5.24	21.28	72.65

Thirty TMRs were evaluated using cluster analysis and five clusters were identified. Second cluster was selected as best on the basis of improved organic matter degradability, total volatile fatty acids, improved microbial biomass production, high metabolizable energy and decreased methane production (Table 7). There are four TMR in this cluster and all the TMR with in a cluster are homogenous. Hence, the two total mixed rations containing rape seed cake and dhanwantharan thailam residue were selected randomly which can be fed to ruminant livestock cost

effectively and with balanced nutrients for efficient growth

Conclusion

The fermentation characteristics of thirty TMRs prepared using unconventional feed ingredients replacing conventional feed ingredients from concentrate mixture and hybrid Napier grass in 50: 50 was evaluated using IVGPT and cluster analysis was done. The TMRs were grouped in to five clusters on the basis of better nutrient digestibility, rumen fermentation profile and lower methane production and the best cluster identified was cluster 2 which includes TMR with dhanwantharam thailam residues from ayurvedic pharmaceutical industry and rape seed cake. It can be concluded that these can be incorporated in the ruminant livestock ration ensuring better nutrient utilization and production and further these can be used as alternate feed resources for formulating low cost rations for ruminants.

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Conflict of Interests

There is no conflict of interest.

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