RESEARCH ARTICLE

In vitro Evaluation of Newer Unconventional Feedstuffs for Livestock

Praveen S. Banakar[1]*, Ally Kulangara[1], Nira M. Soren[3], George Dominic[3], Megolhubino Terhuja[3], Chikkagoudara K. Prasad[2]

Abstract

The study assessed the chemical composition, digestibility, and fermentation metabolites of six unconventional feedstuffs: bamboo leaves (BL), turmeric waste (TW), spent cumin (SC), cooked barley residue (CBR), water hyacinth (WH), and soya sauce waste (SSW) as ruminant feedstuffs through *in vitro* gas production test. The crude protein content ranged from 11.82% (CBR) to 17.98% (WH), while the ether extract was higher in SSW, followed by TW. The neutral detergent fiber (NDF) content was lower in WH (46.6%) and higher in TW (65.83%), while the acid detergent fiber (ADF) was higher in TW and lowered in SSW. Spent cumin had higher lignin than turmeric waste. Gas production (mL/200 mg; 24 h) was higher ($p < 0.05$) in CBR and lowest in the SSW sample. The digestibility of dry matter (DM) and organic matter (OM) followed the same pattern and were higher ($p < 0.05$) in CBR and lower in the BL sample. The microbial biomass production (mg/200 mg DM) was significantly ($p < 0.05$) higher in SSW and lower in CBR and BL samples. The ME (MJ/ kg DM) was higher ($p < 0.05$) for CBR and lower for BL and SSW samples. The NH_3-N (mg/100 mL) concentration was higher ($p < 0.05$) in BL and SSW and lower in other samples. Acetate production was higher ($p < 0.05$) in WH and lowest in other samples, while CBR and WH samples yielded more ($p < 0.05$) propionate than other samples. Total short-chain fatty acids (SCFA) production was higher ($p < 0.05$) in CBR and WH and lowest in other feed samples. Newer feedstuffs like cooked barley residue spent cumin and water hyacinth were highly digestible and rich in energy and therefore can be fed to ruminants.

Keywords: Cooked barley residue, Digestibility, Gas production, *In vitro*, Spent cumin, Turmeric waste, Volatile fatty acids.

Ind J Vet Sci and Biotech (2021): 10.21887/ijvsbt.17.3.17

Introduction

Livestock plays a significant role in the advancement and economic growth of developing countries like India. However, the major constraint associated with livestock production is pressure on the land available for fodder production, which is only 3.3-4.41 % of the total cultivable area (Reddy, 2011). Scarcity of high-cost concentrate feeds and varying quantity and quality of livestock feed for year-round feed supply (Banakar *et al.,* 2017) have led to low productivity, growth, and reproduction of animals. There is a deficit of feed resources in India to the tune of 7.58, 56.73 and 30.37 % for dry roughages, green grasses, and concentrates, respectively (Ramachandra *et al*., 2007). These facts justify the increased use of non-conventional feed resources for animal feeding. Hence, evaluating the nutritive value of different unconventional feeds is of greater importance. The nutritive value of ruminant feed is determined by its chemical compositions and the rate and extent of digestion in the rumen (Kumar *et al*., 2015). Among the available methods, *in vitro* gas production (IVGP) test is one of the best methods for determining the nutritive value of feedstuffs since the rate and extent of degradation and rumen fermentation can be determined by measuring the volume of gas production (Banakar *et al.,* 2017).

In light of the above facts, this study was planned to identify and nutritionally evaluate some of the promising unconventional feedstuffs using *in vitro* gas production test, which would help predict the rumen fermentation characteristics of these feeds and future incorporation of these ingredients more effectively and judiciously in the livestock feeds for optimizing the ruminant productivity.

[1]Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India

[2]Department of LPM, College of Veterinary and Animal Sciences, Pookode, Kerala, India

[3]Animal Nutrition Division, ICAR-National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore, Karnataka, India

Corresponding Author: Praveen Sivakumara Banakar, Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India, e-mail: praveenbanakar24@gmail.com

How to cite this article: Banakar, P.S., Ally, Soren, M., Dominic, G., Terhuja, M., & Prasad, C.K. (2021). *In vitro* Evaluation of Newer Unconventional Feedstuffs for Livestock. Ind J Vet Sci and Biotech, 17(3): 78-83.

Source of support: Nil

Conflict of interest: None.

Submitted: 04/04/2021 **Accepted:** 30/06/2021 **Published:** 16/08/2021

Materials and Methods

Samples Collection and Preparation

Six unconventional feed samples available locally in Kerala were identified and collected for the study. The identified feed samples were bamboo leaves (BL; *Bambusoideae*), turmeric waste (TW; *Curcuma longa*), spent cumin (SC; *Cuminum cyminum*), cooked barley residue (CBR; *Hordeum vulgare*), water hyacinth (WH; *Eichhornia crassipes*) and soya sauce waste (SSW; *Glycine max*). Turmeric waste spent cumin and CBR were collected from ayurvedic medicinal industries located in Thrissur, Kerala. Five kg of all the samples were dried in a hot air oven at 60°C for 48 hours and ground to pass a 1 mm screen, and stored in air-tight containers.

Chemical Analysis

The proximate composition of six unconventional feed samples was determined in duplicate as per AOAC (2005). The cell wall constituents, namely, neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose, and hemicellulose of the feed samples, were determined (Goering and Van Soest, 1970) and expressed inclusive of residual ash. The six feedstuffs' gross energy content was determined using a bomb calorimeter (Parr Instrument Company, USA).

In Vitro Gas Production and Substrate Digestibility

In vitro gas production test (IVGPT) was carried out as per Menke *et al*. (1979). A total of three incubations in non-uplicate (three syringes per incubation) for each sample were kept for assessing 24 hours gas production (GP), digestibility, and other fermentation attributes. For *in vitro* gas production test, the rumen fluid was collected from three 6-year-old male Holstein Friesian crossbred cattle (Average BW 380 ± 15 kg) fitted with a permanent rumen fistula in the morning before feeding. The donor animals were fed on a maintenance diet comprising of green roughage and concentrate mixture @40 and 2 kg/d, respectively. The rumen fluid samples were brought to the laboratory under anaerobic conditions in a thermos flask and strained through a four-layer of muslin cloth, pooled, and used as inoculum for IVGP test.

About 200 mg of samples were weighed into 100 mL calibrated glass syringes in non-uplicate. Petroleum jelly was applied to the piston of the syringes to ease movement and to prevent the escape of gas. The syringes were pre-warmed (39°C) for 1-hours, before the addition of 30 ± 1.0 mL of buffered rumen fluid into each syringe under CO_2 flushing. The syringes were then placed in a BOD incubator at 39°C. Four blank syringes containing only 30 mL of buffered rumen fluid were incubated to estimate gas production due to endogenous substrates for the blank corrections. The syringes were gently shaken every hour during the first 8 hours of incubation, and readings were recorded at the end of 24 hours of incubation. *In vitro* DM and OM digestibility were estimated using methods suggested by Van Soest *et al.* (1991). The metabolizable energy (ME) content of unconventional feedstuffs was calculated using the equation of Menke *et al*. (1979).

Volatile Fatty Acids and Ammonical Nitrogen Estimation

For VFA estimation, an aliquot of 0.8 mL 24 hours post-incubated sub-sample was mixed with 0.2 mL of metaphosphoric acid (25%; w/v) in a micro-centrifuge tube, allowed to stand for half an hour, and centrifuged at 7000 rpm for 20 min at 4°C. The supernatant was injected into gas chromatography (7890A GC System gas chromatography, Agilent Technologies), and individual VFAs were determined as per Filípek and Dvořák (2009). Ammonia nitrogen was determined colorimetrically (Weatherburn, 1967).

Statistical Analysis

The data on *in vitro* gas production, digestibility, and short-chain fatty acids were subjected to one-way analysis of variance using SPSS software (SPSS, 2008). Treatment means were separated using Duncan's multiple range test. A *P-value* of less than 0.05 was accepted to indicate statistical significance.

Results and Discussion

Proximate Composition

The chemical composition and fiber fractions of the evaluated unconventional feed samples are presented in Table 1. The content of non-fiber carbohydrates (NFC) ranged from 2.06 (bamboo leaves) to 25.22% (CBR). The crude protein (CP) in the samples ranged from 11.82 % for CBR to 17.98 % for water hyacinth (WH), while ether extracts ranged from 2.2% in WH to 17.19% in soy sauce waste (SSW). The total ash content ranged from 2.14% in CBR to 26.66% in SSW. The concentration of NDF and ADF was higher in turmeric waste (65.8; 40.7 %), followed by BL and SC. Based on the cell wall constituents, water hyacinth and SSW could be categorized as medium fibrous feeds with high protein content.

The CP content of water hyacinth (WH) found was almost similar to the value reported by Aboud *et al*. (2005) in its leaves (18.03%), shoots (18.04%), and the entire plant (8.53%). The higher CP content of WH plant found is commensurable to some of the common leguminous fodders like cowpea (16.58%), berseem (17.05%) (Kumar *et al.,* 2015). Water hyacinth is also a rich source of minerals with total ash content of 18%, analogous to the value reported by Aboud *et al*. (2005). As such, it contains high moisture (89%); however, before offering to the animal, it can be sun/air dried to reduce the moisture content. It can be fed to a different class of livestock as supplemental feeding to meet the partial protein requirement.

The chemical composition of spent cumin in our study was comparable with values reported by Sultan *et al.* (2009) for black cumin seed. The difference in the value of CP and EE can be attributed to the species difference and extraction process involved during the processing of cumin. The nutritive value of bamboo leaves determined in the present study was in conformity with the finding of Antwi-Boasiako *et al.* (2011) for *Bambusa ventricosa* and *Bambusa vulgaris* leaves. Similarly, Sahoo *et al*. (2009) studied the chemical composition and nutritive value of leaves of different bamboo cultivars and reported that on DM basis, CP ranged from 14.61 to 20.39, EE from 1.39 to 4.73, NDF from 65.38 to 78.53, ADF from 41.58 to 53.23 and AIA from 4.38 to 11.01 %.

In Vitro Gas Production

The average gas productions (mL/200 mg substrate) from the fermentation of different unconventional feeds are shown in Table 2. Gas production ranged from 6.8 mL for SSW to 56.8 mL for CBR. Incubation of cooked barley residue (CBR) yielded a higher ($p < 0.05$) volume of fermentable gas, while soya sauce waste (SSW) was lower among the different samples. The gas production from the incubation of water hyacinth (18.8 mL/200 mg) observed in our study was in agreement with the report (14.5 to 18.5 mL/200 mg DM) of Khan *et al.* (2002). Sahoo *et al.* (2009) in Himachal Pradesh reported gas production ranging from 17.7 to 33.8 mL/200 mg DM for twelve different bamboo leaves at similar maturity levels. These values are higher than the present ones, which may be attributed to the differences in the cell wall content of the selected sample and the seasonal variations.

Digestibility and Metabolizable Energy

The metabolizable energy (MJ/kg DM) contents of different unconventional feedstuffs assessed through *in vitro* gas production test (Table 2) were significantly different ($P < 0.05$) from each other, with the exception of bamboo leaves and water hyacinth. The average ME (MJ/kg DM) of feed samples arranged from highest to lowest was: CBR (11.55), SC (8.1), TW (6.12), SSW (5.3), water hyacinth (4.57), and bamboo leaves (4.52). The predicted ME values of different feedstuffs in our study found were very close to the range reported for other unconventional feedstuffs (Kumar *et al.,* 2015). The differences in the ME value of various unconventional feedstuffs was mostly due to variation in the content of fermentable carbohydrates and nitrogen present in feedstuffs.

The average true DM digestibility (TDMD) percent revealed a significant difference ($p < 0.01$) between BL, TW, SC, CBR WH, but there was no significant difference between SSW, WH, and spent cumin. The TDMD was higher ($p < 0.05$)

Table 1: Chemical composition (% DM basis), fibre and gross energy (kcal/kg) content of unconventional feedstuffs

Component	*BL*	*TW*	*SC*	*CBR*	*WH*	*SSW*
Chemical composition (%)						
Crude protein (CP)	17.41	13.14	17.29	11.82	17.98	17.89
Ether extract (EE)	3.91	12.82	8.31	3.43	2.2	17.19
Crude fibre (CF)	31.33	7.01	14.94	6.28	14.4	11.35
Total ash	16.21	12.0	4.6	2.14	18	26.66
Nitrogen free extract (NFE)	31.14	55.03	54.86	76.33	47.42	26.91
Fibre content (%)						
Neutral detergent fibre (NDF)	60.41	65.83	59.91	57.39	46.59	49.16
Acid detergent fibre (ADF)	30.62	40.70	36.01	27.19	33.95	23.3
Acid detergent lignin (ADL)	13.02	3.47	20.44	3.9	8.31	12.2
Cellulose	20.01	19.33	17.13	13.28	27.6	13.7
Hemicellulose	29.79	25.13	23.90	13.20	12.64	26.03
Non-fibre carbohydrate (NFC)	2.06	3.79	9.89	25.22	15.22	10.90
Gross energy (MJ/kg DM)	14.28	14.69	17.43	15.19	15.28	18.65

BL, Bamboo leaves; TW, Turmeric waste; SC, Spent cumin; CBR, Cooked barley residue; WH, Water hyacinth; SSW, Soya sauce waste.

Table 2: *In-vitro* gas production, digestibility, metabolizable energy, microbial biomass production (MBP) and partitioning factor of unconventional feedstuffs

Attribute	*BL*	*TW*	*SC*	*CBR*	*WH*	*SSW*
Gas production (mL/200 mg; 24 h)	15.57 ± 0.25^{d}	13.5 ± 0.60^{e}	26.42 ± 0.62^{b}	56.75 ± 0.29^{a}	18.8 ± 0.40^{c}	6.82 ± 0.30^{f}
True DM digestibility (%)	49.18 ± 0.33^{e}	60.79 ± 1.20^{d}	76.30 ± 0.56^{b}	87.05 ± 0.26^{a}	72.79 ± 1.36^{c}	74.87 ± 0.31^{bc}
True OM digestibility (%)	49.10 ± 0.62^{e}	60.11 ± 1.20^{d}	76.02 ± 0.57^{b}	86.94 ± 0.26^{a}	71.93 ± 1.62^{c}	74.87 ± 0.10^{b}
Partitioning factor - PF (mg/mL)	5.0 ± 0.07^{d}	7.6 ± 0.20^{b}	5.37 ± 0.14^{cd}	2.87 ± 0.02^{e}	6.02 ± 0.02^{c}	15.5 ± 0.66^{a}
MBP (mg/200 mg DM)	45.37 ± 0.75^{d}	73.52 ± 0.84^{c}	85.15 ± 1.91^{b}	43.52 ± 0.83^{d}	73.55 ± 1.80^{c}	90.57 ± 0.57^{a}
Metabolizable energy (MJ/kg DM)	4.52 ± 0.04^{e}	6.12 ± 0.10^{c}	8.1 ± 0.10^{b}	11.55 ± 0.05^{a}	4.57 ± 0.06^{e}	5.3 ± 0.05^{d}

Means with different letters in a row differ significantly ($p < 0.05$).
BL, Bamboo leaves; TW, Turmeric waste; SC, Spent cumin; CBR, Cooked barley residue; WH, Water hyacinth; SSW, Soya sauce waste.

in CBR and lower in BL. The trend of true OM digestibility (TOMD) among the different unconventional feed followed a similar pattern to that of TDMD, and accordingly, a significant difference ($p < 0.01$) was observed between all the feed samples except for SSW (74.87 ± 0.10) and spent cumin (76.02 ± 0.57). Accordingly, the TOMD of CBR was significantly ($p < 0.05$) higher and that of BL was lower in the study. The value obtained in the present study for WH was 26 % higher than that reported by Khan *et al.* (2002). The higher value obtained in our study was attributed to lower fiber and ADL levels in the substrate. The estimated values of digestibility of twelve bamboo leaves by Sahoo *et al.* (2009) ranged from 47.43 to 56.45% for dry matter and 50.58 to 63.26% for organic matter. The TDMD and TOMD of bamboo leaves in the present study are in agreement with these reported values. The relatively low content of structural fiber in the feedstuffs can facilitate the colonization of the feed by the rumen microbial population, which in turn might induce higher fermentation rates, therefore improving digestibility. A lower level of structural carbohydrates (NDF and ADF: Table 1) and higher content of NFC in CBR gave rise to higher DM and OM digestibility in the present study.

Microbial Biomass Production and Partitioning Factor

Results pertaining to the microbial biomass production (MBP) and partitioning factor (PF) are presented in Table 2. The MBP was significantly higher ($p < 0.05$) in SSW and lower in BL and CBR. However, there was no significant difference between TW (73.52 mg) and WH (73.55 mg), and BL (45.37 mg), and CBR (43.52 mg). Microbial nitrogen is the major source of protein for ruminants and is utilized to meet the maintenance requirement of the animal. Microbial biomass is produced in the rumen from the degradation of feed material (substrate). In the *in vitro* gas production system, the substrate (200 mg) kept for incubation is either degraded by the rumen microbes into short-chain fatty acids or incorporated into microbial biomass with the liberation of fermentation gases. Microbial biomass production is more when a substrate is used to grow micro-organisms and less for the fermentative gas. Water hyacinth and turmeric waste could be helpful in the synthesis and supply of microbial protein.

According to Blümmel *et al.* (1999), higher PF value of a feed indicates higher efficiency of MBP and lower methane output by the ruminant. The partitioning factors for SSW (15.5), turmeric waste (7.6 ± 0.20) and water hyacinth (6.02 ± 0.02) were more than the theoretically possible value of 4.41. The highest MBP production of these three unconventional feed samples concurred with results of Blümmel *et al.* (1999). Thus, these feed samples might also be helpful in mitigating methane production. The higher PF has been reported for tannins-rich feedstuffs which ranged from 3.1 to 16.1 (Getachew *et al.,* 2000). The high PF could be either due to the solubilization of tannins from the feed, and these tannins would not make any contribution to gas or energy in the system. Furthermore, cooked barley residue (2.87 ± 0.02) had the lowest PF value, followed by spent cumin and bamboo (5.37 ± 0.14 and 5 ± 0.07 respectively). The PF is considered as an index of efficiency of microbial biomass synthesis (Blümmel *et al.,* 1999). Proportional to the amount of substrate degraded, lower gas production (higher PF) is an indicator of greater microbial biomass synthesis (Singh *et al.,* 2010).

Fermentation Metabolites

Data related to total volatile fatty acids (TVFA; mmol/L), acetate: propionate ratio and ammonical nitrogen of the evaluated feeds is presented in Table 3 and individual VFA in Fig. 1. Rumen ammoniacal nitrogen production was highest ($p < 0.05$) in BL and SSW and lower in other feedstuffs without any significant difference among them. Eschenlauer *et al.* (2002) suggested that the ammonia concentration is mainly

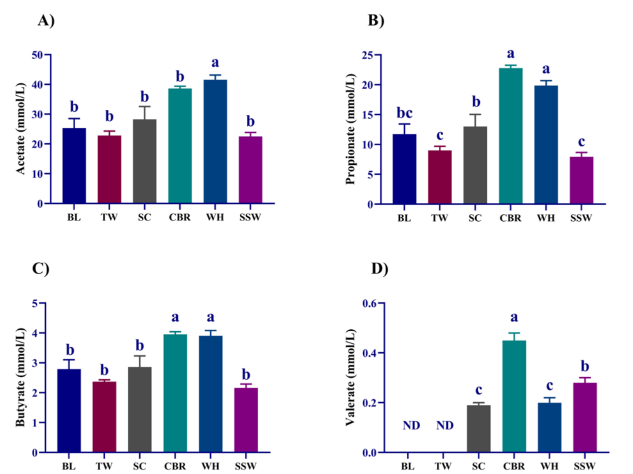

Fig. 1: Individual volatile fatty acids (mmol/L): A) Acetate, B) Propionate, C) Butyrate, D) Valerate, production from different unconventional feedstuffs. Columns with different letters in a graph differ significantly ($p < 0.05$).
BL, Bamboo leaves; TW, Turmeric waste; SC, Spent cumin; CBR, Cooked barley residue; WH, Water hyacinth; SSW, Soya sauce waste; ND, Not detected.

Table 3: *In vitro* ruminal ammonical nitrogen and total volatile fatty acids production from different unconventional feedstuffs

Attribute	*BL*	*TW*	*SC*	*CBR*	*WH*	*SSW*
Ammonical nitrogen (mg/100 mL)	37.01 ± 4.25^{a}	18.15 ± 0.89^{b}	18.80 ± 0.85^{b}	17.09 ± 0.40^{b}	19.07 ± 2.11^{b}	31.30 ± 2.42^{a}
Total volatile fatty acids (mmol/L)	39.87 ± 5.17^{b}	34.18 ± 2.26^{b}	44.30 ± 6.75^{b}	65.76 ± 1.38^{a}	65.52 ± 2.60^{a}	32.90 ± 2.18^{b}
C2:C3 ratio	2.19 ± 0.08^{c}	2.54 ± 0.05^{b}	2.18 ± 0.01^{c}	1.69 ± 0.02^{d}	2.01 ± 0.01^{c}	2.87 ± 0.11^{a}

Means with different letters in a row differ significantly ($p < 0.05$).
BL, Bamboo leaves; TW, Turmeric waste; SC, Spent cumin; CBR, Cooked barley residue; WH, Water hyacinth; SSW, Soya sauce waste.

related to the content of protein in the feed samples and also it is influenced by the factors that have effect on the degradability of the proteins. The same phenomenon was evident in our study with higher concentration of ammonia production in BL and SSW samples vis-à-vis the other samples. The higher production of ammonia in bamboo leaves indicated a higher level of soluble protein in it.

The concentration of acetate (mmol/L) was significantly ($p < 0.05$) higher in water hyacinth (40) and CBR (38.59) than in soya sauce waste, bamboo leaves, turmeric waste and spent cumin (22.54, 25.37, 22.81 and 28.29, respectively). Hungate *et al*. (1952) reported that the rations containing large amounts of cellulose are characterized by high numbers of gram-negative cocci, which are responsible for more acetate production. The same phenomenon was evident in this study for water hyacinth (27.6% cellulose) sample and the lower production of acetate in SSW may be due to less cellulose available (13.7%) for fermentation.

The propionate concentration (mmol/L) was higher ($p < 0.05$) in CBR (22.78) and water hyacinth (19.87) and lower in rest of the feedstuffs (Fig. 1). Cooked barley residue had highest NFC content which is the main precursor for propionate production. Degradation of organic matter into VFA results in the formation of surplus hydrogen gas. Under most condition the surplus of H_2 is eliminated by methanogenic bacteria (Van Lingen *et al*., 2016). Under conditions of a high rate of degradation of non-structural carbohydrates, the capacity of methanogenic bacteria may be insufficient to remove the surplus of hydrogen. Thus, over accumulation of hydrogen in the rumen is prevented by the microflora diverting the production of acetate to more reduced acids such as propionate and lactate) Ungerfeld, 2015). Similar phenomenon might have enhanced the propionate production in some of the feed samples in the present study. The NFC content was high for CBR (25.22) and water hyacinth (15.22), which acts as a main precursor for propionate synthesis. When a large quantity of non-structural carbohydrates is fed to the animal, a large proportion of the TVFA formed in the rumen is propionate than when large amount of structural carbohydrates are present in the diet.

The concentration of butyrate (mmol/L) was higher ($p < 0.05$) in CBR (3.95) and water hyacinth (3.90) than rest of the feedstuffs. Valeric acid was not detected in the incubation media of bamboo leaves and turmeric waste. Valerate concentration (mmol/L) was higher ($p < 0.05$) in CBR (0.45) than in SSW (0.28), water hyacinth (0.20) and spent cumin (0.19). The total volatile fatty acids (mmol/L) concentration followed similar pattern to that of butyrate concentration and was higher ($p < 0.05$) for CBR (3.95) and water hyacinth (3.90 ± 0.18) and lower for rest of the feedstuffs.

The acetate to propionate ratio was wide ($p < 0.05$) in SSW (2.87) and significantly narrow in rest of the feeds. The narrower ratio indicated more propionate production thus less hydrogen is available for methane production, and a wide-ratio is an indication of a more acetogenic fermentation associated with the activity of fibrinolytic bacteria degrading substrates rich in structural carbohydrates (Blümmel *et al*., 1999).

Conclusion

Nutrient composition of water hyacinth, spent cumin, bamboo leaves, and soya sauce waste showed that these feedstuffs could be useful as protein supplements in ruminant feeding. The enhanced values of metabolizable energy and volatile fatty acid in cooked barley residue spent cumin and water hyacinth imply its ability to meet the energy requirements of tropical ruminants. However, protein supplementation will be required when turmeric waste and cooked barley residue are included in the diet for ruminants. Nevertheless, more research, on animal responses, through *in vivo* experiments is required to support the nutritional characteristics reported in this study.

Acknowledgements

The authors are thankful to the Director, ICAR-NIANP, Bangalore, for providing the necessary facilities to work in Feed Quality and Safety Laboratory. The financial support in the form of institutional scholarship for MVSc. The first author duly acknowledges thesis research work from KVASU.

References

Aboud, A. A. O., Kidunda, R. S., & Osarya, J. (2005). Potential of water hyacinth (*Eicchornia crassipes*) in ruminant nutrition in Tanzania. *Livestock Research for Rural Development*, 17(8), 205.

Antwi-Boasiako, C., Coffie, G. Y., & Darkwa, N. A. (2011). Proximate composition of the leaves of *Bambusa ventricosa, Oxytenanthera abyssinica* and two varieties of *Bambusa vulgaris*. *Scientific Research and Essays*, 6(34), 6835-6839.

AOAC. (2005). Official Methods of Analysis. 18th ed. *Association of Official Analytical Chemists*, Arlington, Virginia, USA.

Banakar, P., Ally, K., Lokesh, E., Saseendran, A., Dominic, G., & Jaafar, J. (2017). *In vitro* assessment of nutritive value of unconventional feed resource as livestock feed. *International Journal of Livestock Research, 7*, 159-169.

Blümmel, M., Aiple, K. P., Steingaβ, H., & Becker, K. (1999). A note on the stoichiometrical relationship of short chain fatty acid production and gas formation *in vitro* in feedstuffs of widely differing quality. *Journal of Animal Physiology and Animal Nutrition, 81(3)*, 157-167.

Blümmel, M., Karsli, A., & Russell, J. R. (2003). Influence of diet on growth yields of rumen micro-organisms *in vitro* and *in vivo*: influence on growth yield of variable carbon fluxes to fermentation products. *British Journal of Nutrition, 90(3)*, 625-634.

Eschenlauer, S. C. P., McKain, N., Walker, N. D., McEwan, N. R., Newbold, C. J., & Wallace, R. J. (2002). Ammonia production by ruminal micro-organisms and enumeration, isolation, and characterization of bacteria capable of growth on peptides and amino acids from the sheep rumen. *Applied and environmental microbiology, 68(10)*, 4925-4931.

Filípek J and Dvořák R. (2009). Determination of the volatile fatty acid content in the rumen liquid: Comparison of gas chromatography and capillary isotachophoresis. *Acta Veterinaria Brno 78*: 627-33.

Getachew G, Makkar H P and Becker K. (2000). Effect of polyethylene glycol on *in vitro* degradability of nitrogen and microbial protein synthesis from tannin rich browse and herbaceous legumes. *British Journal of Nutrition*, *84*(1), 73-83..

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.

Hungate R E, Dougherty R W, Bryant M P and Cello R M. (1952). Microbiological and physiological changes associated with acute indigestion in sheep. *Cornell veterinarian, 42*, 423-449.

Khan M J, Steingass H and Drochner W. (2002). Evaluation of some aquatic plants from Bangladesh through mineral composition, *in vitro* gas production and *in situ* degradation measurements. *Asian-Australasian Journal of Animal Science 4*, 537-542.

Kumar D, Datt C, Das L K and Kundu S S. (2015). Evaluation of various feedstuffs of ruminants in terms of chemical composition and metabolisable energy content. *Veterinary World, 8*, 605-609.

Menke K H, Raab L, Salewski A, Steingass H, Fritz D and Schneider W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor. *Journal of Agricultural Science, 93***,** 217–222.

Paquay R, Godeau J M, De Baere R and Lousse A. (1973). Utilization of nutrients by the dairy cow and optimal N: energy ratio in the diet. *Journal of Dairy Research, 40*, 329-37.

Ramachandra K S, Taneja V K, Anandan S and Angadi U B. (2007). Livestock feed resources in different agro ecosystems of India: availability, requirement and their management. National Institute of Animal Nutrition and Physiology, Bangalore, India. pp.100.

Reddy D V. (2011). Advanced animal nutrition. Oxford & IBH Publishing Company Pvt. Ltd, New Delhi, India. pp. 37.

Sahoo A, Ogra R K, Sood A and Ahuja P S. (2009). Chemical composition and nutritive value of leaves from different bamboo cultivars. *Indian Journal of Animal Nutrition, 26,* 306-14.

SPSS. (2008). Statistical package for social sciences, Statistics for windows, version 17. Released 2008. Chicago, USA.

Sultan M T, Butt M S, Anjum F M, Jamil A, Akhtar S and Nasir M. (2009). Nutritional profile of indigenous cultivar of black cumin seeds and antioxidant potential of its fixed and essential oil. *Pakisthan Journal of Botany, 41*, 1321-30.

Ungerfeld, E. M. (2015). Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: a meta-analysis. *Frontiers in Microbiology, 6*, 37.

Van Lingen H J, Plugge C M, Fadel J G, Kebreab E, Bannink A and Dijkstra J. (2016). Thermodynamic driving force of hydrogen on rumen microbial metabolism: a theoretical investigation. *PLOS One, 111*, 1-18.

Van Soest P J, Robertson J B and Lewis B A. (1991). Methods of dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74,* 3583–3597.