International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(2): 873-876

Received: 19-01-2020
Accepted: 21-02-2020

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Evaluation of spent coffee waste as a ruminant feed by *in vitro* gas production technique

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DOI: https://doi.org/10.22271/chemi.2020.v8.i2m.8875

Abstract
Aim of the present study was to evaluate the feeding value of spent coffee waste in ruminants. Nutrient composition of spent coffee waste (SCW) revealed that it contains Crude protein, Ether extract, Crude fibre and Nitrogen free extract at 12.02%, 9.23%, 56.20% and 21.73% respectively. SCW had higher neutral detergent fibre, acid detergent fibre and Lignin content of 79.67, 68.54 and 34.08% respectively when compared with conventional feeds and also contained high amount of Acid detergent insoluble protein (67.54%). Total phenol content in SCW was 0.35%. *In vitro* gas production studies revealed that SCW has significantly (P<0.01) lower gas production potential (2.13 ml), *in vitro* true dry matter digestibility (17.48%) and ammonia nitrogen (1.75 mg/100ml) content when compared with conventional feeds like maize. It can be concluded that spent coffee waste as such has less potential as a concentrate feed source in ruminants.

Keywords: Spent coffee waste, crude protein, *In vitro* dry matter digestibility, phenol

Introduction
India possesses world's highest livestock population (15.06% cattle and 57.83% buffaloes) and produces 146.3 million tons (18.5% of world) of milk. Though India is the highest milk producer in the world, the productivity of Indian dairy animals is still below their genetic potential (BAHS, 2015) [2]. A major constraint to increase the productivity is the scarcity and availability of conventional feed resources. In India, there is an acute deficit of conventional feeds *viz.*, 35.6% green fodder, 10.95% dry fodder and 44% concentrate feed ingredients (IGFRI, 2015) [7]. Due to ever increasing human population, grazing lands and area under fodder cultivation are dwindling fast because they are mostly diverted for cultivation of commercial crops to meet the human needs. Hence, animal nutrition research is more focused on alternative feed resources to replace the conventional feeds in order to meet the nutrient demand of livestock as well as to reduce the feed cost.
In India, much attention has been paid on by-products from processing of crops and food products as alternative feed resources because of their constant availability, environmental benefit besides reducing the cost of production (Venkateswarlu *et al*., 2013) [18]. Spent coffee waste (SCW) is one such by product obtained during the extraction of instant coffee from coffee beans. It is a mixture of coffee pulp and husk and constitutes about 40% of the whole beans. With increasing international coffee trade, amount of spent coffee waste production has also increased. If it is not disposed properly, it causes environmental pollution (Choi *et al.*, 2018) [4]. Coffee by products are rich, low cost source of carbohydrates, proteins and bio active compounds such as caffeine, polyphenols and melanoidin might have beneficial effect on animals (Vignoli *et al.*, 2011) [20]. Several studies have been conducted to use SCW for biodiesel production, compost and sorbent metal ion removal. However, scanty literature is available regarding its usefulness as animal feed. Hence the present study was aimed to evaluate the nutrient composition, Phenolic compounds, *in vitro* dry matter digestibility and rumen fermentation characteristics of spent coffee waste under *in vitro* conditions.

Materials and Methods
Spent coffee waste (SCW) samples were collected from instant coffee beverage factory CCL products limited (Machikalapudi village, Andhra Pradesh).

SCW were dried in hot air oven at 60 ^{0}C and ground in a Wiley mill to pass through a 2 mm sieve. SCW was analyzed for Crude protein, Ether Extract, Crude fibre and total ash according to AOAC (2007) [1]. Organic matter was calculated as weight loss during ashing at 550 ^{0}C. Fibre fractions like Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Cellulose, Acid detergent lignin (ADL) were analyzed as per Van Soest *et al.*, (1991) [17].

Estimation of secondary plant Metabolites

Total phenols (TP) and total tannins (TT) in the extract were determined by Folin-Ciocalteu method (Makkar 2003) using polyvinylpolypyrrolidone to separate the non tannin phenols from total phenols, and condensed tannins (CT) were determined by the butanol–HCl–iron method. The hydrolysable tannins (HT) were calculated by difference between TT and CT (Singh *et al.*, 2005). Total phenols and tannins were expressed as tannic acid equivalent and CTs as leucocyanidin equivalent.

***In vitro* gas production studies**

The *in vitro* gas production studies were carried out using Hohenheim gas production technique (Menke and Steingass, 1988) [10]. Rumen liquor samples were collected from three crossbred cattle maintained on paddy straw, Co-4 grass and concentrate, under anaerobic conditions. The samples were collected in the morning before feeding and watering. About 200 mg of sample was incubated with 30 ml of buffered rumen inoculum in 100 ml calibrated glass syringes and were kept in automatic water bath shaking incubator set at 39 ^{0}C. At the end of incubation period (24 h), the total gas produced was measured and the gas samples were collected in vaccutainer for estimation of methane using gas chromatography (Perkin Elmer, Clarus 500 model) equipped with Flame Ionization Detector (FID). Fermented fluids were collected for estimation of ammonia nitrogen (Makkar and Becker 1996) [8]. The pH of the rumen liquor was measured immediately after end of incubation by using Corning electronic pH electrode. *In vitro* true dry matter digestibility was estimated as per the procedure of Van Soest and Robertson (1988) [16].

Statistical analysis of the data was carried out with analysis of variance (ANOVA) as per Snedecor and Cochran (1994) [15] using SPSS version 20.0.

Results and Discussion

Chemical composition of spent coffee waste expressed on dry matter basis (DMB) is presented in table 1. Chemical composition revealed that SCW contains mostly organic matter (99.18%) and very little amount of inorganic matter (0.82%). Crude protein content in SCW was 12.02% and it is within the range of values reported by various authors (Choi *et al.*, 2018, Rahimnejad *et al.*, 2015 and Sikka *et al.*, 1985) [4, 11, 13]. Ether extract content is 9.23% and lower than that reported by sikka *et al*., 1985 [13] and XU *et al.*, 2007 [21]. However Fornaroli and Perotti (1976) [5] reported that ether extract content may vary according to technology used in the processing of coffee beans and it is in the range of 1.4% - 26%. High amount of crude fibre (56.20%) and low level of soluble carbohydrates (21.73%) in SCW was observed in the present study. It might be attributed to the complete extraction of soluble fractions during the processing of the beans (Sikka *et al.*, 1985) [13].

Table 1: Chemical Composition (on % DMB) of spent coffee waste

Nutrients	Percent
Dry matter	29.94 ± 0.07
Organic matter	99.18 ± 0.03
Crude protein	12.02 ± 0.19
Ether Extract	09.23 ± 0.64
Total Ash	0.82 ± 0.03
Crude Fibre	56.20 ±1.32
Nitrogen free Extract	21.73 ± 1.23
NDF	79.67 ± 0.71
ADF	68.54 ± 0.69
Cellulose	33.17 ± 0.60
ADL	34.08 ± 0.65
Hemicellulose	11.13 ± 0.96
Silica	1.29 ± 0.12
NDICP (% of Crude Protein)	74.38 ± 1.35
ADICP (% of Crude Protein)	67.54 ± 1.69

Each value is mean of six observations

Phenolic composition of spent coffee waste is presented in table 2. The total phenol content is 0.35% out of which 2/3rd was non tannin phenols and 1/3rd was tannin phenols. The tannins in SCW were mostly in the form of hydrolysable tannins and tannins will have effect on reducing methane emission from ruminants (Venkateswarlu *et al.* 2018) [18]. Coffee Phenolics have potential antioxidant and free radical scavengers and quantity observed in present study is similar to that of Rahimnejad *et al.* (2015) [11].

Table 2: Secondary plant metabolites in spent coffee waste on percent dry matter basis

Phenolic compounds	Percent on dry matter basis
Total Phenols	0.35 ± 0.03
Non Tannin Phenols	0.24 ± 0.01
Tannins Phenols	0.11 ± 0.03
Hydrolysable Tannins	0.10 ± 0.03
Condensed tannins	0.01 ± 0.01
Each value is mean of six observations	

Effect of Spent Coffee waste on total gas (ml), methane (ml), *in vitro* true dry matter digestibility, methane (ml) per 100 mg of truly digested substrate, pH and Ammonia nitrogen is presented in table 3. Levels of total gas production, methane and *In vitro* true dry matter digestibility (%) were significantly (P<0.01) lower compared with conventional feeds. This is possibly due to the non-enzymatic chemical reaction between amino acids and reducing sugars (Maillard reaction) which occur during roasting of beans, thereby decreasing the nutrient digestibility (Bekedam, 2008) [3]. Hence limited amount of SCW could be used as ruminant feed due to low protein, dry matter digestibility and palatability (Xu *et al.*, 2007 and Givens and Barber 1986) [21, 6].

Table 3: Effect of Spent Coffee waste on total gas (ml), methane (ml), *in vitro* true dry matter digestibility, methane (ml) per 100 mg of truly digested substrate, pH and Ammonia-nitrogen

Parameter	SCW	Maize	Soybean meal
Total gas production (ml/200mg)	$2.13^{c} \pm 0.17$	$41.89^{a} \pm 0.45$	$35.63^{b} \pm 0.57$
Methane (ml)	$0.18^{c} \pm 0.02$	$12.26^{a} \pm 0.16$	$8.39^{b} \pm 0.14$
% Methane on total gas production	$8.63^{c} \pm 0.21$	$29.26^{a} \pm 0.27$	$23.55^{b} \pm 0.16$
Methane (ml) for 100 mg of truly digested substrate	$0.53^{c} \pm 0.05$	$7.80^{a} \pm 0.11$	$5.36^{b} \pm 0.10$
In vitro true dry matter digestibility (%)	$17.48^{b} \pm 0.51$	$78.59^{a} \pm 0.33$	$78.31^{a} \pm 0.58$
pH	$6.02^{b} \pm 0.04$	$6.28^{b} \pm 0.03$	$6.69^{a} \pm 0.03$
Ammonia Nitrogen (mg/100ml)	$1.75^{c} \pm 0.30$	$9.63^{b} \pm 0.35$	$22.52^{a} \pm 0.42$
Each value is mean of six observation Means bearing different superscripts in a same row differ significantly (P<0.01)			

Rumen pH values were below the normal level due to acid nature of coffee waste. Hence it may cause sub acute acidosis if proper care is not taken while feeding coffee waste. Xu *et al.*, (2007) [21] also reported decreased rumen pH when wet coffee grounds silage was used at 10 and 20% level in total mixed rations. Ammonia nitrogen content (mg/100ml) was 1.75 and it was nearer to the values reported by Seo *et al.*, (2015) [12]. Low level of gas production, methane and *in vitro* dry matter digestibility indicated that spent coffee waste inhibited the rumen fermentation.

Conclusion

Spent coffee waste having medium protein content of 12.02%. However, most of the protein is in the form of unavailable fraction which indicates availability of protein to animals in SCW is very low. Further, *In vitro* dry matter digestibility and total gas production of SCW were also low indicating spent coffee waste inhibiting the rumen fermentation. Hence it could be concluded that spent coffee waste as such may not provide adequate nutrients for ruminants and also having less scope as a feed source for ruminant feed. However, further *in vivo* studies need to be carried out to ascertain feeding value of spent coffee waste in ruminant's rations.

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