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Research Article

IN VITRO RUMEN FERMENTATION KINETICS AND MICROBIAL BIOMASS SYNTHESIS OF UNCONVENTIONAL SUGARCANE TRASH AND CONCENTRATES USING CATTLE INOCULUMS

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Abstract- The unconventional dry fodder sugarcane trash (SCT) obtained after harvest of sugarcane was treated with four per cent urea for ammonisation and the samples of SCT, urea ammoniated sugarcane trash (USCT) and concentrates (GMG & CFM) was subjected for chemical analysis. The proximate composition and fibre fraction of SCT was within the range when compared to other crop residues. The in vitro gas production at 24 h and predicted metabolizable energy (ME) of SCT was lower than USCT. The t½ of roughage source reduced for USCT than SCT, whereas concentrates had lowest t½ than roughage. The Partitioning factor (PF), microbial biomass production (MBP) and efficiency of microbial biomass synthesis (EMBS) at t½ were higher in USCT when compared to SCT. Hence, urea ammoniated sugarcane trash can be recommended as roughage source to ruminants, due to improved ME, truly digested organic matter (TDOM), PF, MBP and EMBS when compared to untreated SCT.

Keywords- Sugarcane trash, urea, *in vitro*, gas production

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INTRODUCTION

A major constraint to livestock production in India is the scarcity of both green and dry fodder. The unconventional feeds are by-product feedstuffs used for feeding livestock which are obtained during harvesting or processing of agriculture produce. Ruminants are fed low quality crop residues in various proportions depending on the type of animal and season. These crop residues are poor in nutritional value. India stands second in the world in sugarcane production, Karnataka on 4th position with 4.1 lakh hectare cultivable area. Sugarcane trash is crop residue left over on fields after harvesting sugarcane. The quantity of sugarcane trash available is around 3- 6 tons/hectare after harvest [1]. It is burnt on sugarcane field due to rough surface area, sharp leaf edges and woody nature. It has low feeding value and poor palatability of trash but contains higher fibre fractions. Therefore, to improve the nutritive value, SCT can be treated with urea for ammonisation which makes the material soft and pliable and further the samples subjected for in vitro gas production. In vitro gas production technique has been used to determine the rate and extent of dry matter degradation. Since in vitro methods are less expensive, less time consuming, allow more control of experimental conditions than in vivo experiments. Volatile fatty acids, amino acids, microbial protein and gases are produced during fermentation process by microbes of rumen [2]. The microbial protein produced in rumen by micro-organisms is the major source of protein for ruminants and prediction of efficiency of microbial protein production is very important in ruminant nutrition. The objective of the study was to compare the gas production
kinetics, metabolizable energy; organic matter digestibility and microbial protein production of untreated sugarcane trash with urea ammoniated sugarcane trash and concentrates (GMG & CFM).

Materials and Methods

The experiment was conducted at department of animal nutrition, Veterinary College, Bidar. Sugarcane trash was procured from the sugarcane field at Bidar after harvest of sugarcane. The trash was chopped and four per cent urea with 40 per cent water used to enrich the chopped sugarcane trash then kept air tight for 21 days for urea ammonisation. The representative samples of SCT, USCT, ground maize grain (GMG) and formulated concentrate feed mixture (CFM) were analysed for proximate principles and fibre fraction then the samples were subjected to rumen in vitro gas production technique (RIVGPT) according to the procedure described by Menke and Steingass, (1988) [3] to estimate the metabolisable energy content of feedstuff. The rate and volume of gas production was estimated from the cumulative gas production at incubation time varying from 2 to 96 h by means of nonlinear regression to know the kinetics of gas production.

Donor animal and collection of rumen fluid

A Deoni bull weighing 250 kgs aged 3 years, fitted with a flexible rumen canula of large diameter (Bar Diamond, Inc. USA), receiving a basal diet consisting of sorghum stover and concentrate feed mixture (Maize-50 %, Soybean meal-20.5 %, Wheat bran-25 %, DCP -2.0, Mineral mixture-1.0 %, Salt-0.75 %, Vitamin-0.25 %, Sodium bi carbonate-0.5 %) was used for the donor animal for rumen fluid. The sorghum stover and concentrate were fed separately; the sorghum stover was offered 3 kg in small portions 4 times in a day, starting at 9.30 AM.

The CFM was offered 1.0 kg per day in two equal portions at 6 AM and 1.30 PM. Drinking water was offered three times a day and 2 hours before rumen fluid collection. Rumen fluid was collected in the morning between 9.00 to 9.30 AM before offering roughage.

Metabolisable energy (ME) determination

The ME content of SCT, USCT and concentrates were determined by rumen in vitro gas production technique (RIVGPT) according to procedure described by Menke and Steingass, (1988) [3] using the following equations:

Concentrate feed:
ME = 1.06 + 0.1570 GP +0.0084 CP + 0.022 EE -0.0081 TA

Roughages:
ME = 2.2 +0.1357 GP + 0.0057 CP + 0.0002859 EE2

Where, GP = gas production (ml/200mg DM); CP, EE, TA are crude protein, ether extract and total ash, respectively, in g/kg DM.
ME= Metabolisable energy, MJ/kg DM

Kinetics of gas production

Air equilibrated feed samples (200±10 mg) of SCT, USCT and concentrates were incubated in 100 ml calibrated glass syringe in triplicate with 30 ml buffered rumen fluid with three blank incubations. The incubation was done in water bath maintained at 39˚C. The readings of displaced syringes were recorded at different time intervals 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60, 72, 84 and 96 h of incubation. Whenever the syringe readings exceed at 90 ml, the readings were reset to 30 ml, and then cumulative gas production for 96 h time period was calculated. For determination of ME content of test samples, 24 h net cumulative gas production was used (corrected for blank and reference standard) at 24 h of incubation [16]. The rate and extent of gas production were calculated by non-linear regression using the model $Y = D\ (1 - e^{-k*t})$ where, Y is gas volume (ml) at time t, D is potential gas production (ml) and k is rate (per hour) at which gas is produced [4]. The time at half asymptotic gas production (t1/2) was calculated as ln2/k.

Microbial biomass synthesis

The microbial biomass synthesis of SCT, USCT and concentrates was calculated by determining the ratio of TDOM and gas production at the time at which half maximum gas production was achieved ($t_{1/2}$) as described by Blummel, *et al*., (1997) [2]. One set of incubation was kept to determine PF values at $t_{1/2}$ of incubation. Three replicates of 500 ± 10 mg of air equilibrated feed samples were weighed into 100 ml calibrated syringes and incubated with 40 ml of mixed rumen suspension at 390C with parallel incubations of blanks. Incubations were terminated by recording gas production at t1/2 for the respective feed samples by immersing in ice water bath to prevent further microbial activity. The contents of the syringes were quantitatively transferred through the nozzle of the syringe into 600 ml spoutless Berzelius beakers. The syringes were rinsed with 100 ml neutral detergent solution by dispensing 25 ml neutral detergent solution into the syringe each time. Refluxed the incubation residue for 1 h followed by filtration on preweighed gooch crucibles to recover true undigested matter [5]. Crucibles with undigested residue were dried at 100^{0}C overnight weighed to determine true undigested residue. Residue was ashed at 500^{0}C for 3 hours to determine true undigested organic matter. The TDOM was calculated as difference between OM incubated and undigested OM of feed origin recovered in the residue. The PF was calculated as ratio of mg TDOM to ml gas produced at $t_{1/2}$.

Results and discussion

Chemical composition of sugarcane trash

The detailed chemical composition of SCT, USCT and concentrates were presented in [Table-1]. The results of the proximate analysis and forage fibre fractions of sugarcane trash is compared to values reported by [6]. The CP and total ash content of sugarcane trash in the present study was high, lignin and cellulose content was low, whereas the hemicelluose content was similar to the reported values of Franco, *et al*., (2013) [6]. The chemical composition of sugarcane trash was within range when compared to other crop residues as reported by several authors in wheat straw [7], finger millet straw [8] and rice straw [9]. The ADL content in sugarcane trash was high when compared to other crop residues.

Table-1 *Chemical composition (% on DMB) of SCT, USCT and Concentrates*

Composition	SCT	USCT	GMG	CFM
Dry matter	92.7	74.53	91.85	91.18
Organic matter	88.63	86.6	93.07	92.07
Crude protein	3.04	11.98	10.45	29.72
Ether extract	1.46	1.7	1.87	1.96
Crude fibre	34.73	34.17	1.1	2.11
Nitrogen free extractives	49.4	38.74	79.65	58.27
Total ash	11.37	13.4	6.93	7.93
Acid insoluble ash	11.62	9.73	1.35	0.68
Neutral detergent fibre	79.09	78.8	16.49	18.96
Acid detergent fibre	50.27	52.65	4.79	7.72
Acid detergent lignin	20.75	17.27	0.61	0.66
Cellulose	29.29	33.07	3.18	5.78
Hemicellulose	28.82	26.15	11.71	11.24
Gas production-24 (ml/g DM)	101.5	172	387.8	319.2
Metabolizable energy (MJ/kgDM)	5.2	7.6	14	13.4

The CP, ADF and cellulose content of USCT increased but decreased in CF, NFE, NDF, ADL and hemicelluloses content was observed when compared to SCT. Marginal increase in CP content of USCT was due to addition of urea as NPN source for ammonisation of SCT and decrease in NDF, ADL and hemicelluose was due to solubilisation of hemicellulose during ammonisation as reported by Horton, (1981) [10]. Reduction in ADL content of USCT was due to solubilization of lignin and the disruption of the intermolecular ester bonds between uronic acid cellulose and hemicelluloses [11]. The results of the study were well correlated with the findings of Horton and Steacy, (1979) [12] for barley, wheat, and oat straw. Improved CP content upon urea treatment in rice straw [13] and in wheat straw [14]. The untreated SCT, is equivalent to any of the cereal crop residue like paddy or wheat straw, bajra or maize stover in its chemical composition.

In vitro gas production

Data of gas production (ml/200mg DM) at different intervals of incubation periods by RIVGPT are given in [Table-2] and the same graphically represented in [Fig-1]. The cumulative gas production increased during incubation period. The in vitro gas production was gradual and maximum in concentrates than roughage source. SCT has lower net gas production than USCT. This may be due to higher digestibility of USCT than SCT similarly concentrates are highly digestible hence higher gas production recorded.

Predicted metabolisable energy (ME) by RIVGPT

The in vitro gas production at 24 h (GP-24, ml/g DM) and predicted metabolizable energy (ME, MJ/kg DM) of SCT was lower than USCT. This could be attributed to higher lignification in SCT and lower lignin and higher digestibility of USCT upon ammoniation which increased substrate availability to rumen microbes, when compared to SCT. Higher content of nitrogen in USCT resulted in increased gas production, it was in agreement with study conducted by Nsahlai, *et al*., (1994) [15] there was positive correlation between crude protein content and rate of gas production. Negative correlations between neutral detergent fibre (NDF) and acid detergent fibre (ADF), and the rate and extent of gas production. Predicted ME value of SCT was similar to ME value of paddy straw, and that of USCT was close to ME values of sorghum stover, maize stover and finger millet straw [16].

Gas production kinetics

The t½ (h) of roughage source reduced for USCT when compared to SCT [Table-3]. Whereas GMG and CFM had lowest t½ value when compared to SCT or USCT. This was due to higher availability of nutrients and higher digestibility for concentrates followed by USCT when compared to SCT.

Rate of gas production k (h^{-1}) and potential gas production D (ml/g DM) were lower for SCT than for USCT. Higher k and D value observed for concentrates. This indicated that increase in rate of gas production linearly increased potential gas production and similarly the Gas at $t_{1/2}$ (ml). The results were similar to the observations reported by Blummel, *et al*., (2003) [17] for soybean meal, maize grain, lucerne hay, oat berseem clover hay and maize crop residue. Anup Kumar, (2016) [16] for maize stover, sorghum stover, paddy straw, fingermillet straw, maize husk and concentrate.

Table-2 The mean net gas production (ml/200mg DM) at different intervals of time (h) by RIVGPT

Incubation time (h)	SCT	USCT	GMG	CFM
2	2.63	2.8	4.64	7.69
4	4.74	3.95	15.87	18.6
6	7.38	7.91	31.74	29.17
8	9.84	11.86	43.87	37.57
10	11.6	15.81	50.5	42.58
12	12.82	18.33	55.85	45.99
16	15.45	24.08	63.34	51.89
20	17.02	28.57	68.51	56.18
24	18.78	31.81	71.72	59.05
30	21.77	35.58	75.47	61.91
36	24.23	38.64	78.32	63.88
48	27.4	41.87	80.99	65.13
60	31.26	44.75	83.31	67.1
72	32.85	46.01	84.2	67.46
84	34.96	47.62	85.1	68.35
96	36.19	47.98	84.92	68.71

Table-3 *Gas production kinetics (potential gas production (D, ml/g DM), rate of Gas production (k h^{-1})), substrate degradation (truly digested OM (TDOM, mg/g DM)), partitioning factor (PF, mg TDOM/ml gas at $t_{1/2}$), microbial biomass production (MBP, mg) and efficiency of microbial biomass synthesis (EMBS, g/kg TDOM) for the SCT, USCT, GMG and CFM*

Particulars	SCT	USCT	GMG	CFM
Kinetic parameters				
$t_{1/2}$ (h)	25.85	14.58	6.817	6.824
k (h^{-1})	0.0268	0.0475	0.1017	0.1016
D (ml)	192.35	240.5	413.85	334
Gas at $t_{1/2}$ (ml)	55.57	45.61	59.13	58.92
Substrate degradation (mg/g DM) at t1/2				
TDOM at $t_{1/2}$	335.3	325.8	505.1	675.4
Microbial biomass synthesis indices at t1/2				
PF at $t_{1/2}$ (mg/ml)	2.82	3.33	3.95	5.27
MBPa at $t_{1/2}$ (mg)	73.5	110.7	205.7	375.2
EMBSb at $t_{1/2}$ g/kg)	218.5	339.4	405.3	555.4

[a]*MBP= [TDOM – (gas at t1/2 x SF)], where SF (stoichiometric factor) was considered as 2.20 for roughage and 2.34 for CFM supplements*
[b]*EMBS = (MBP/TDOM) x 1000*

Substrate degradation (TDOM) and Microbial biomass synthesis indices

The TDOM values at t½ were higher for CFM when compared to roughage [Table-3]. This was due to lower content of hemicellulose, cellulose, and lignin in CFM when compared to roughage SCT and USCT. The PF, MBP and EMBS at t½ were higher in USCT when compared to SCT. CFM had higher values than roughage source [Table-3]. Whereas, CFM had higher values when compared to GMG. The USCT had a higher initial rate of gas production than SCT indicated that it was more readily digested by the rumen microbes and improved microbial biomass synthesis indices. The GMG and CFM were highly digestible than roughage which had higher gas production and higher PF value. This indicated that higher MBP and EMBS [18]. The PF values were in agreement with the values of various feed stuffs reported by Blummel, *et al*., (1997); Krishnamoorthy, *et al*., (2005) [19], [20], and Anup Kumar, (2016) [16].

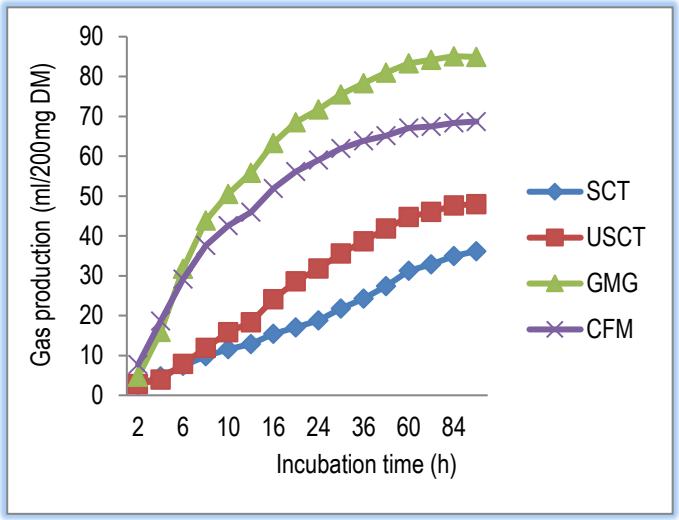

Fig-1 Rate of gas production (k) of SCT, USCT,GMG and CFM

Summary and Conclusion

The nutritive value of SCT was improved when SCT treated with 4 per cent urea. SCT, USCT, GMG, CFM were subjected to rumen in vitro gas production (RIVGP) technique. The t½ (h) in roughage source reduced for USCT when compared to SCT. Whereas CFM had lowest t½ value when compared to roughage source. k (h^{-1}) and D (ml/g DM) were lowest for SCT when compared to USCT. Whereas higher k and D value observed for CFM. The TDOM, PF, MBP and EMBS at t½ were higher in USCT when compared to SCT. GMG and CFM had higher values than roughage source. The unconventional sugarcane trash can be treated with urea for ammonisation to improve the nutritive value of trash as USCT had improved ME, TDOM, PF, MBP and EMBS when compared to untreated SCT. The roughage source with higher microbial biomass synthesis indices in vitro indicate higher digestibility. Hence, urea ammoniated sugarcane trash can be recommended as roughage source to ruminants, especially for stallfed sheep and goat, due to improved ME, TDOM, PF, MBP and EMBS when compared to untreated SCT. However, in vivo experiment is needed to check palatability and to be more informative about unconventional sugarcane trash.

Application of research

This in vitro study helps to initiate use of unconventional sugarcane trash as roughage source to feed livestock. Further animal experiments need to be conducted to check the palatability, digestibility and performance of animals.

Research Category: *in vitro* gas production, rumen kinetics

Abbreviations:

SCT: Sugarcane trash
USCT: Urea ammoniated sugarcane trash
GMG: Ground maize grain
CFM: Concentrate feed mixture
ME: Metabolizable energy
PF: Partitioning factor
MBP: Microbial biomass production
EMBS: Efficiency of microbial biomass synthesis
TDOM: Truly digested organic matter
SF: Stoichiometric factor
RIVGP: Rumen in vitro gas production

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