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Rumen fermentation kinetics and microbial biomass synthesis of super Napier silage, CofFS29 Silage, Tur Pods and concentrates by *in vitro* gas production technique

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Abstract
The super napier silage, CoFS 29 silage, tur pods and concentrate feed mixture (CFM) procured and subjected for chemical analysis. Estimated proximate composition of silage was within the range when compared to other grass silages variety. The *in vitro* gas production and predicted metabolizable energy for 24 hours is higher in CFM and lower in tur pods. Among the silages, CoFS 29 silage had higher *in vitro* gas production and predicted metabolizable energy for 24 hours. The t½ was lower in CoFS 29 silage when compared to super napier silage. The partitioning factor, microbial biomass production, efficiency of microbial biomass synthesis were higher in CoFS 29 silage when compared to super napier silage and tur pods. Hence, CoFS 29 silage can be used as a good quality roughage source over Super napier silage and Tur pods due to improved truly digestible organic matter, energy value, partitioning factor and microbial biomass synthesis.

Keywords: super napier silage, CoFS 29 silage, Tur pods, *In vito* gas production, ME

1. Introduction
The livestock's growth and development is conditioned by the adequate availability of feed and fodder. Forages usually constitute the major portion of the ruminant feeds and high quality nutritious green fodder is required to exploit the productive potential of dairy animals (Datta, 2013) [8]. Apart from sufficient unavailability of green fodders in our country, inconsistent quality of fodder is another major constraint in tropical countries, particularly in the summer affecting ruminant production to a great extent (Jamsawat *et al.*, 2017) [11]. Therefore, cultivation of newer verities of perennial fodders with higher biomass per unit area and preserving excess forage as silage is an immediate solution to overcome the shortage of forage to meet the demand of current livestock production and ensure nutricious green fodder could be made available throughout the year (Khaini *et al.*, 2015) [13]. Super napier fodder and CoFS 29 a multicut sorghum fodder variety (TNS 30 x Sorghum sudanense) fodder are high yielding verities, their fodders produced need to be conserved as silage to prevent the loss of nutrients to the possible extent and make use in lean season for maintaining the animal productivity. Tur pods can be used as dry roughage along with the green to increase the dry matter intake. Hence, the present experiments have been designed to evaluate super napier silage, CoFS 29 silage, tur pods and concentrates for its rumen fermentation kinetics and microbial biomass synthesis by *in-vitro* gas production technique.

2. Materials and Methods
Super napier and CoFS 29 fodders were sourced from Livestock Research & Information Center (Deoni), Hallikhed (B), Bidar. Super napier and CoFS 29 fodders were freshly cut and chopped to 1-2 inch size before silage preparation. Chaffed fodders were filled into silo bags, made air tight and stored for a period of 45 days. Concentrate feed mixture (CFM) and tur pods were procured from the department of ILFC, Veterinary College, Bidar. The experiment was conducted at department of Animal Nutrition, Veterinary College, Bidar. The representative samples were subjected for estimation of proximate principles (AOAC, 2016) [4] and fibre fractions (Van Soest *et al.* 1991) [24]. The dried samples of super napier silage, CoFS 29 silage and CFM were ground to 1mm size and subjected to rumen *in vitro* gas production

technique (RIVGPT) according to the procedure described by Menke and Steingass, (1988) [16] 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 hours by means of nonlinear regression to know the kinetics of gas production

2.1 Donor animal and collection of rumen fluid
Rumen liquor was collected from the adult Kenguri sheep (Average body weight of approximately 25kg) using stomach tube. Sheep used for rumen fluid collection was fed with chaffed sorghum stover *ad-lib* as a basal diet. Additionally, concentrate was quantified and offered twice a day in equal proportion to meet the maintenance requirement (ICAR 2013) [9]. 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 9.30AM before offering roughage in thermos flask.

2.2 Metabolisable energy (ME) determination
The ME content of super napier silage, CoFS 29, tur pods and CFM were determined by rumen *in vitro* gas production technique (RIVGPT) according to procedure described by Menke and Steingass, (1988) [16] 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

2.3 Kinetics of gas production
Air equilibrated dried feed samples (200±10 mg) of super napier silage, CoFS 29 silage, tur pods and CFM 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 (0. 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60, 72, 84 and 96 hour) of incubation. Whenever the syringe readings exceed the 90 ml, the readings were reset to 30 ml and then cumulative gas production for 96 hour was calculated. 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). The time at half asymptotic gas production (t1/2) was calculated as ln2/k.

2.4 Microbial biomass synthesis
The microbial biomass synthesis of super napier silage, CoFS 29 silage, tur pods and CFM was calculated by determining the ratio of TDOM and gas production at half maximum gas production was achieved (t1/2) Blummel, *et al*., (1997) [6]. One set of incubation was kept to determine PF values at t1/2 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 39 ^{0}C. Incubations were terminated by recording gas production at t1/2 for the respective feed samples by immersing in ice water bath to arrest microbial fermentation. The contents of the syringes were quantitatively transferred into 600 ml spout less 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 one hour followed by filtration on preweighed gooch crucibles to recover true undigested matter. Crucibles with undigested residue were dried at 100 ^{0}C overnight weighed to determine true undigested residue. Residue was made ash 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 t1/2.

3. Results and Discussion
3.1 Chemical composition
Proximate composition of silages, tur pods and CFM were presented in the Table 1. The OM, CP and EE values of super napier silage were comparable with values of super napier silage reported by Kaewpila *et al.* (2020) [12] whereas NDF, ADF values were lower and ADL values was higher in the present study. The DM, EE, CF, NFE, NDF, ADF and ADL percent values are comparable with the value reported by Markos (2015) [15]. In the present study, the CP content of super napier silage was 7.17 per cent. Similar values for CP was reported in napier grass silage by earlier workers (Nurjana *et al.* 2016) [20]. In contrast, lower (Rego *et al*. 2010) [22] and higher (Saha *et al.* 1994; Aganga *et al*, 2005) [23, 1] CP content was also reported as compared to the CP content observed in the present study.

Table 1: Proximate composition of super napier silage, CoFS 29 silage, tur pods and CFM (DMB)

Composition	Super Napier silage	CoFS 29 silage	Tur pods	CFM
DM	20.54	29.95	92.09	94.88
OM	90.42	91.51	94.87	77.03
CP	7.17	6.94	8.44	14.12
EE	1.85	1.46	0.95	1.82
CF	30.06	26.93	34.11	5.26
NFE	51.34	56.18	51.37	55.83
TA	9.58	8.49	5.13	22.97
AIA	4.48	4.79	1.74	9.76
NDF	63.34	59.84	51.51	22.88
ADF	39.30	33.86	35.22	10.23
ADL	7.85	7.53	9.75	3.86
Cellulose	36.57	35.82	26.69	8.67
Hemicellulose	24.04	25.98	16.29	12.64
GP-24 (ml/g DM)*	186.28±2.68	204.36±2.90	165.56±4.37	269.52±7.10

ME (MJ/kg DM)*	8.16±0.04	8.14±0.07	7.35±0.09	11.03±0.12

Note : All the values were means of three observations
* Mean of six observations from two incubation

The DM, CP, CF, EE, TA NFE, ADL, cellulose and Hemicellulose, values for CoFS 29 silage where highly correlating with the values reported by Senthilkumar *et al.* (2009) whereas NDF and ADF values were lower in the present study. The NDF and ADF values where comparable to the values of sorghum forage reported by the Wadhwa *et al.* (2010) [25]. In the present study, the CP content of CoFS 29 silage was 7.06 per cent. Similar values for CP were reported in multi-cut sorghum varieties by earlier workers (Panwar *et al*., 2000; Wadhwa *et al.*, 2010; Muragan *et al.*, 2002) [21, 25, 18]. In contrast, lower (Miron *et al.* 2005) [17] and higher (Iyanar *et al.,* 2016; Ramya *et al.*, 2017) CP content was also reported as compared to the CP content observed in CoFS 29 silage.

In the present study, percent DM and CP is higher in the CFM followed by the tur pods, Super napier silage and CoFS 29 silage. Similarly percent CF is lower in the CFM followed by the, CoFS 29 silage and Super napier silage. Tur pods contain higher CF(%) and lignin(%) than the other. Other all parameters are comparable to each other.

3.2 *In vitro gas* production

The *in vitro* gas production at 24 hour GP-24 (ml/g DM) in tur pods, super napier silage, COFS 29 silage and CFM were linearly increased. CFM had higher gas production compare to silages whereas tur pods contain lower gas production (Table 2). High fermentable carbohydrates containing feeds easily digest with higher rate of gas production (Anup Kumar, 2016; Jaishankar *et al.*, 2018) [3, 10]. The CFM contains highly fermentable carbohydrates than the roughages, hence higher *in vitro* gas production observed. Silages are partially fermented product, the nutrients are easily digested with higher rate of gas production whereas the tur pod is dried crop residue harvested after complete maturation of the crop, the lignin content is higher that affect digestibility (Anna Karkonen, *et al.*, 2014) [2], hence the less gas production.

Table 2: *In vitro* gas production (ml) of super napier silage, CoFS 29 silage, tur pods and CFM.

Incubation time(h)	Super Napier Silage	CoFS 29 Silage	Tur pods	CFM
2	3.8	4.3	3.8	7.7
4	7.6	7.8	6.5	13.8
6	11.6	11.4	8.9	20.3
8	17.5	19.3	13.1	29.4
10	22.5	24.8	17.4	32.8
12	26.5	28.8	20.4	38.2
16	30.3	32.1	24.9	44.8
20	34.4	35.8	28.0	47.9
24	38.2	40.6	32.3	51.3
30	41.2	43.9	35.3	51.0
36	43.6	45.7	36.8	51.1
48	48.0	48.6	39.8	52.5
60	49.9	51.7	42.7	52.8
72	51.2	52.4	43.1	52.7
84	52.7	53.9	44.9	53.1
96	54.5	55.0	45.8	54.0

3.3 Predicted metabolisable energy (ME) by RIVGP

The *in vitro* gas production at 24 h (GP-24, ml/g DM) and predicted metabolizable energy (ME, MJ/kg DM) of super napier silage was higher than tur pods and lower than the CoFS 29 silage. This could be attributed to higher lignification and CF in tur pods and lower lignin and higher digestibility of silages which increased substrate availability to rumen microbes, when compared to tur pods. Higher content of soluble sugars in silages resulted in increased gas production; it was in agreement with study conducted by Nsahlai, *et al*., (1994) [19].

3.4 Gas production kinetics

The t½ (h) of tur pods is highest when compared to silages (Table- 3), whereas, CFM had lowest t½ value when compared to silage and tur pods. This was due to higher availability of nutrients and higher digestibility for concentrates followed by CoFS 29, super napier silage and tur pods.

Rate of gas production k (h-1) and potential gas production D (ml/g DM) were found to be lower for tur pods and higher for CFM. Similarly, rate of gas production k (h-1) and potential gas production D (ml/g DM) were higher for CoFS 29 when compare to super napier silage. This indicated that increase in rate of gas production linearly increased potential gas production and similarly the gas at t1/2 (ml). The results were similar to the observations reported by Blummel, *et al*., (2003) [6] for soybean meal, maize grain, lucerne hay, oat berseem clover hay and maize crop residue. Anup Kumar, (2016) [3] for maize stover, sorghum stover, paddy straw, fingermillet straw, maize husk and concentrate. Jaishankar *et al.,* (2018) [10] for unconventional roughage source like sugarcane trash and urea ammoniated sugarcane trash.

3.5 Substrate degradation (TDOM) and Microbial biomass synthesis indices

The TDOM, PF, MBP and EMBS values at t½ were significantly (<0.01) 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. The CFM were highly digestible than roughage which had higher gas production and higher PF value. As PF is the ratio of truly degraded organic matter and gas produced, is an indication of higher efficiency of microbial biomass (Blummel *et al*., 1997a) [6]. The PF, MBP and EMBS at t½ were higher in CoFS 29 than the super napier silages and significantly lower (<0.01) in tur pods. The silages had a higher initial rate of gas production than tur pods indicated that silages were more readily digested by the rumen microbes and improved microbial biomass synthesis indices. The PF, MBP and EMBS values were in agreement with the values of various feed stuffs reported by Blummel, *et al*., (1997b) [7]; Krishnamoorthy, *et al*., (2005) [14], Anup Kumar, (2016) [3] and Jaishankar *et al.*, (2018) [10].

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 t1/2), microbial biomass production (MBP, mg) and efficiency of microbial biomass synthesis (EMBS, g/kg TDOM) of super napier silage, CoFS 29 silage, tur pods and Concentrate feed mixture(CFM)

Particulars	Super Napier Silage	CoFS 29 Silage	Tur pods	CFM
Kinetic parameters				
t1/2 (h)	12.65	11.41	13.64	6.62
k (h-1)	0.05479	0.06075	0.05082	0.1047
D (ml)	263.6	267.00	225.40	267.00
Gas at t1/2 (ml)	41.29^{c}± 0.86	48.98^{b}± 1.23	36.81^{d}± 1.10	55.22^{a}± 0.51
t1/2 (h)	12.65	11.41	13.64	6.62
Substrate degradation (mg/g DM) at t1/2				
TDOM at t1/2	307.91^{cb} ± 7.67	365.28^{b}± 9.31	260.71^{c}± 6.58	533.30^{a}± 10.26
Microbial biomass synthesis indices at t1/2				
PF at t1/2 (mg/ml)	3.43^{bc}± 0.08	3.71^{b}± 0.07	3.07^{c}± 0.11	4.78^{a}± 0.13
MBP at t1/2 (mg)	50.51^{b}± 3.15	73.89^{b}± 3.10	29.81^{c}± 3.56	134.57^{a}± 5.87
EMBS at t1/2 (g/kg)	356.24^{bc}± 10.80	406.32^{b}± 10.67	262.90^{c}± 27.89	508.98^{a}± 12.97
ME MJ /DM	8.16^{b} ± 0.04	8.14^{b}± 0.07	7.85^{c}± 0.04	11.03^{a}±0.12

Note: P<0.01, Mean values bearing different superscript in a row differ significantly

4. Conclusion

The samples of CoFS 29 silage, super napier silage, tur pods and CFM were subjected to rumen *in vitro* gas production (RIVGP) technique. The t½ (h), k (h-1) and D (ml/g DM) of CoFS 29 and super napier silage are better than tur pods but lower than concentrate. The TDOM, PF, MBP and EMBS at t½ were higher in silages when compared to tur pods. The metabolizable energy of silages was higher than tur pods and lower than the CFM. The CoFS 29 silage with higher microbial biomass synthesis indices *in vitro* indicate higher digestibility than the super napier silage, however, both silages were higher in value of EMBS than the tur pods. Hence, CoFS 29 silage is better than super napier silage but both better than tur pods therefore, can be recommended as better roughage source to ruminants, especially for stall fed sheep and goat, due to improved ME, TDOM, PF, MBP and EMBS. However, *in vivo* experiment is needed to check acceptance of super napier and CoFS 29 silage and blood biochemical profile to ascertain growth and production performance in ruminants.

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