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Evaluation of sugarcane trash based complete diets for rumen degradation kinetics and microbial biomass synthesis by *in vitro* gas production technique

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Abstract

Sugarcane trash (SCT) is the unconventional crop residue almost burnt on the sugarcane field after the harvest of canes due to poor palatability. The concept of feeding complete rations or total mixed ration with use of locally available crop residues seems to be ideal and promising method of improving the utilization of poor quality fibrous crop residues. Therefore, SCT procured, grinded and formulate six complete diets with various proportion of roughage: concentrate (D-1 80:20, D-2 70:30, D-3 60:40, D-4 50:50, D-5 40:60 and D-6 30:70) further subjected for chemical analysis and RIVIGPT to assess the quality of diets based on *in vitro* gas production kinetics, metabolisable energy (ME), truly digestible organic matter (TDOM), partitioning factor (PF), microbial biomass production (MBP) and efficiency of microbial biomass synthesis (EMBS). The *in vitro* gas production at 24 h (GP-24, ml/g DM), predicted ME (MJ/kg DM), TDOM, PF, MBP and EMBS for complete diets increased linearly with increase in the proportion of concentrate feed mixture (CFM) from 20 to 60 per cent in the diet. Marginal reduction in PF, MBP and EMBS was observed with 70 per cent of CFM supplemented diet. The optimum microbial biomass indices were observed at D-4 diet with 50% of SCT and marginal reduction over 60% of SCT in diet was observed. Hence, it is recommended that SCT can be incorporated upto the level of 50% in complete diet for efficient utilization. However, *in vivo* experiment is required to check palatability and to validate *in vitro* results and to be more informative about unconventional sugarcane trash.

Keywords: sugarcane trash, RIVIGPT, *in vitro*, gas production, partitioning factor

1. Introduction

Efficient utilization of crop residues is one of the thrust area in ruminant nutrition to mitigate the shortage of feed resources to meet the maintenance requirements of livestock. Ruminants are fed on low quality crop residues in various situations. 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 (Jaishankar *et al*., 2017) [10]. It is burnt on sugarcane field due to rough surface area, sharp leaf edges, low feeding value and poor palatability of trash but contains higher fiber fractions. Therefore concept of feeding complete rations or total mixed ration (TMR) with use of locally available poor palatable crop residues seems to be ideal and promising method of improving the utilization of poor quality fibrous crop residues, because it prevents selection of ingredients during feed consumption. Metabolisable energy (ME) content predicts the quality of feed and fodder hence metabolisable energy content of feed or diets were estimated by conducting digestion trial in animals. But conducting animal trials is time consuming, laborious, expensive and requires large quantity of feeds which is not suitable for large scale feed evaluation. Therefore several alternative methods were proposed to conventional digestion trial and the Hohenheim *in vitro* gas test is widely accepted and adopted technique in routine feed evaluation to predict *in vivo* digestibility and metabolisable energy contents of feeds (Menke and Steingas, 1988) [14] Since, this technique being less expensive and less time consuming, but they allow one to maintain experimental conditions accurately than do *in vivo* trials. The aim of this experiment was to assess *in vitro* gas production kinetics, metabolizable energy, partitioning factor, organic matter digestibility and microbial protein production of various proportion of sugarcane trash based complete diets.

2. 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 grinded sugarcane trash used as roughage source for formulation of six complete diet of various roughage: concentrate ratio (D-1 80:20, D-2 70:30, D-3 60:40, D-4 50:50, D-5 40:60 and D-6 30:70). The representative samples of diet were analysed for proximate principles and fiber fraction. Further, the samples subjected to rumen *in vitro* gas production technique (RIVGPT) according to the procedure described by (Menke and Steingass, 1988) [14] 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.

2.1 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 stovers and concentrate were fed separately; the sorghum stovers 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.

2.2 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) [14] using the following equations:

Concentrate feed

ME = 1.06 + 0.1570 GP +0.0084CP+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 feed samples (200±10 mg) of complete diets 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 at 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 (Menke and Steingass, 1988) [14].

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 (Krishnamoorthy *et al*., 1991) [11] The time at half asymptotic gas production (t1/2) was calculated as ln2/k.

2.4 Microbial biomass synthesis

The microbial biomass synthesis of sugarcane trash based complete diets was obtained by determining the ratio of truly digested organic matter (TDOM) and gas production at the time at which half maximum gas production was achieved ($t_{1/2}$) as described by (Blummel *et al.,* 1997a) [5]. One set of incubation was kept to determine PF values at $t_{1/2}$ of incubation. 0 at $t_{1/2}$ for the respective diet 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 (Goering and Vansoest., 1970) [8]. Crucibles with undigested residue were dried at 100 ºC overnight weighed to determine true undigested residue. Residue was ashed at 500 ºC 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 $t_{1/2}$

3. Results and Discussion

3.1 Chemical composition of sugarcane trash based complete diet

The chemical composition (g/kg DM), *in vitro* gas production at 24 h (GP-24, ml/g DM) and predicted ME (ME, MJ/kg DM) content six complete diets is presented in Table 1. The inclusion of higher proportion of CFM in the diet lead to linear increased in OM, CP, EE and NFE but decreased linearly CF, TA, AIA, NDF, ADF, ADL, cellulose and hemicellulose content of the diet. The same increasing trend observed in gas production and predicted metabolisable energy content of the diets. This was due to the higher or lower content of respective nutrients in the concentrate supplements and the SCT used for formulation of complete rations. Similar results in complete rations with various roughage source were reported by Reddy and Prasad (1983) [19] with cowpea hay, Cheeke, (1999) [6], Gomes *et al.* (2004) with wheat straw, Assefu (2012) [4] with sward hay, Venkateswarlu *et al.* (2014) [21] with sorghum stover and Nalini *et al.* (2013) [16] with sweet sorghum bagasse as roughage source.

Table 1: Chemical composition (% on DMB) of complete diets

Parameter	D-1	D-2	D-3	D-4	D-5	D-6
DM	95.86	95.88	96.44	95.43	96.70	96.66
OM	87.10	87.86	87.48	88.97	88.64	88.13
CP	9.41	11.17	12.08	12.35	13.22	13.12
EE	0.939	1.041	1.152	1.633	1.706	1.966
CF	28.28	27.47	26.37	25.03	18.39	16.03
NFE	48.47	48.18	47.88	49.96	55.32	57.01
TA	12.90	12.14	12.52	11.03	11.36	11.87
AIA	7.91	6.84	6.51	5.64	4.34	4.07
NDF	76.85	72.04	68.30	60.45	54.58	50.37
ADF	48.88	44.63	42.78	35.08	31.26	28.07
ADL	21.17	19.80	18.64	17.95	16.00	14.29
Cellulose	28.11	27.24	26.29	25.56	23.80	22.65
Hemi- cellulose	27.97	27.41	25.52	25.37	23.33	22.29
Gas prodn-24h (ml/g DM)	104.1	122.6	137.2	176.7	216.6	244.3
ME (MJ/kgDM)	5.3	5.9	6.2	7.5	8.6	9.4

3.2 *In vitro* gas production

Data of *in vitro* 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 linearly increased with increase in proportion of concentrates in the diet. This might be due to highly digestibility of concentrates than sugarcane trash hence higher gas production recorded.

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

Diet	2	4	6	8	10	12	16	20
80:20	2.56	4.94	7.83	8.69	10.22	11.59	14.48	17.21
70:30	3.41	6.31	8.86	11.08	13.47	14.83	17.73	20.62
60:40	3.43	7.20	10.80	13.20	15.77	17.66	20.57	23.49
50:50	5.13	8.91	13.53	17.80	21.05	23.28	27.21	30.46
40:60	5.72	12.62	19.02	23.56	27.60	29.80	34.00	37.54
30:70	6.09	14.22	21.66	26.91	31.14	34.19	38.76	42.15

Diet	24	30	36	48	60	72	84	96
80:20	19.26	21.81	23.69	26.76	29.48	30.85	32.04	32.73
70:30	22.67	25.40	27.62	30.51	33.58	34.77	35.97	37.16
60:40	25.37	27.95	29.83	32.24	34.81	35.83	37.21	37.89
50:50	32.68	35.07	37.13	40.20	42.94	44.31	45.51	46.19
40:60	40.07	43.09	45.28	48.48	50.83	51.68	52.85	53.70
30:70	45.19	48.24	50.61	53.32	55.52	56.53	57.89	58.73

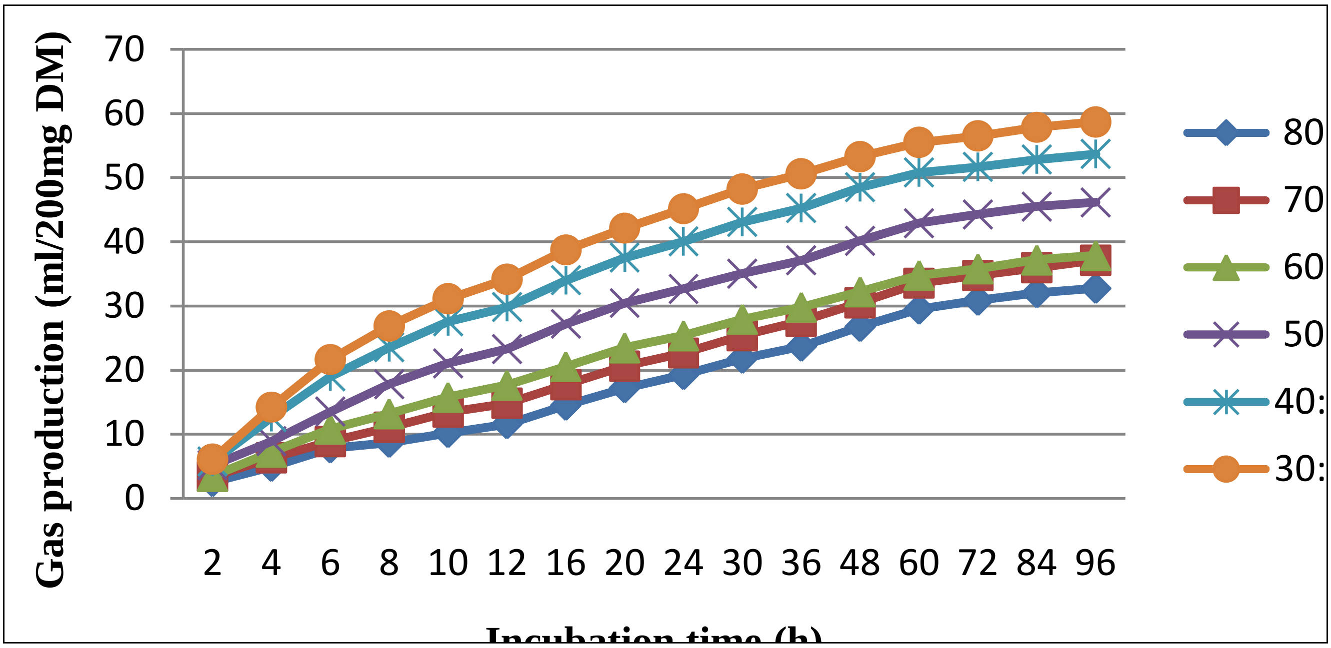

Fig 1: rate of gas production (k) of complete diets

3.3 Predicted metabolisable energy (ME) by RIVGPT
The *in vitro* gas production at 24 h (GP-24, ml/g DM) and predicted ME (ME, MJ/kg DM) for complete diets increased linearly with increase in the proportion of CFM in the diet (Table 1). This was due to increase in the nutrient availability to rumen microorganisms for microbial fermentation (Ahmed and Abdel, 2007) [1]; Mahala and Elseed (2007) [13]. Similar results were observed by Nalini *et al.* (2012) [15] for sweet sorghum bagasse based complete diet, Ramana reddy *et al.* (2015) [17] for sorghum stover based complete diets, Anup kumar (2016) [3] for finger millet straw based complete diet and Ramana reddy *et al.* (2016) [18] for Maize stover based complete diets.

3.4 Gas production kinetics
The $t_{½}$ (h) in complete diet reduced gradually with increase in proportion of CFM in diets (Table 3). Whereas k (h^{-1}), D (ml/g DM) and Gas production at $t_{1/2}$ (ml) linearly increased with increase in the proportion of CFM in diet. This was due to availability of better digestible nutrients from CFM for rumen microbes. Similar results were observed and reported by Anup kumar (2016) [3] with finger millet straw based complete diets.

Table 3: Gas production kinetics (potential gas production (*D*, ml/g DM), rate of Gas production (*k* h^{-1})), substrate degradation (TDOM, mg/g DM), partitioning factor (PF, mg TDOM/ml gas at $t_{1/2}$ and 24 h), microbial biomass production (MBP, mg) and efficiency of microbial biomass synthesis (EMBS, g/kg TDOM) for complete diets

Particulars	D-1 (80:20)	D-2 (70:30)	D-3 (60:40)	D-4 (50:50)	D-5 (40:60)	D-6 (30:70)
Kinetic parameters						
t1/2 (h)	20.94	18.82	14.83	13.04	10.87	9.751
k (h-1)	0.0331	0.0368	0.0467	0.0532	0.0638	0.0712
D (ml)	169.75	187.55	186.30	225.35	259.95	282.75
Gas at t1/2 (ml)	37.64	43.21	48.57	60.08	64.99	67.51
Substrate degradation (mg/g DM) at t1/2						
TDOM at t1/2	290.8	340.7	385.8	480.2	514.8	519.3
IVDMD at t1/2 (%)	37.6	41.9	46.8	54.9	59.4	61.0
Microbial biomass synthesis indices at t1/2						
PF at t1/2 (mg/ml)	3.73	3.79	3.82	3.86	3.87	3.73
MBP[a] at t1/2 (mg)	117.0	139.1	157.6	197.7	211.1	199.3
EMBS[b] at t1/2 (g/kg)	402.1	408.3	408.5	411.6	410.0	383.6

[a]MBP= [TDOM – (gas at $t_{1/2}$ x SF)], where SF (stoichiometric factor) was considered based on roughage and CFM proportion in the diets.
[b]EMBS = (MBP/TDOM) x 1000

3.5 Substrate degradation (TDOM) and Microbial biomass synthesis indices
The TDOM values at $t_{½}$ was linearly increased with gradual increase in proportion of CFM in the complete diets (Table 3). The increased TDOM values was due to gradual reduction in hemicellulose, cellulose, and lignin content in the diet with increased proportion of CFM, which act as limiting factors to lower the digestibility at excess amount of fiber fractions. Improved TDOM with increased proportion of CFM in the ration supports more nutrient availability to microorganism's (Al-Masri, 2009) [2], similar results were observed and reported by Nalini *et al.* (2012) [15] for sweet sorghum bagasse based total mixed ration, Ramana reddy *et al.* (2015) [17] for sorghum stover based complete diets, Ramana reddy *et al.* (2016) [18] for Maize stover based complete diets and Anup kumar (2016) [3] for finger millet straw based complete diets.
The IVDMD values at t ½ were linearly increased with gradual increase in proportion of CFM in the diet. This was due to nutritive content and higher digestibility of CFM than roughage. Similar results were also observed at 24 h of incubation by Elghandour *et al.* (2015) [7] and Kumar *et al.* (2013) [12].

3.6 Microbial biomass synthesis indices
The PF, MBP and EMBS at $t_{½}$ for complete diets linearly increased with gradual increase in the proportion of CFM from 20 to 60% in the diet (Table 3). Whereas marginal reduction in PF, MBP and EMBS was observed with 70% CFM supplemented diet. This was due to changed microbial population due to highly fermentable nutrients supplied through CFM that affect fermentation. Highly digestible nutrients present in CFM indicated higher PF, MBP and EMBS values when linearly increased in complete diets. The results were in agreement with the results observed and reported by several authors Nalini *et al.* (2012) [15] and Seshaiah *et al.* (2014) [20] with sweet sorghum bagasse based total mixed ration; Ramana Reddy *et al.* (2015) [17] with sorghum stover based complete diets; Ramana Reddy *et al.* (2016) [18] with Maize stover based complete diets and Anup Kumar (2016) [3] with finger millet straw based complete diet.

4. Conclusion
The *in vitro* results of unconventional sugarcane trash based complete diets reveal that, the *in vitro* gas production at 24 h (GP-24, ml/g DM), predicted ME (MJ/kg DM), TDOM, PF, MBP and EMBS for complete diets increased linearly with increase in the proportion of CFM from 20 to 60 per cent in the diet. Marginal reduction in PF, MBP and EMBS was observed with 70 per cent of CFM supplemented diet. The optimum microbial biomass indices were observed at D-4 diet with 50% of SCT and marginal reduction over 60% of SCT in diet was observed. Hence, it is recommended that SCT can be incorporated upto the level of 50% in complete diet for efficient utilization. However, *in vivo* experiment is required to check palatability and to validate *in vitro* results and to be more informative about unconventional sugarcane trash.

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6. References
1. Ahmed GN, Abdel NM. Chemical composition and *in vitro* gas production characteristics of six fodder trees

leaves and seeds. J Agric. Biol. Sci 2007;3:983-986.
2. AL-Masri MR. An *in vitro* nutritive evaluation and rumen fermentation kinetics of *Sesbania aculeata* as affected by harvest time and cutting regimen. Trop. Anim. Health Prod 2009;41:1115-1126.
3. Anup Kumar PK. A study on the effect of fibrolytic enzymes on the *in vitro* rumen fermentation, feed intake, nutrient digestibility and lactation performance in crossbred dairy cattle. Phd thesis, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, India 2016.
4. Assefu Gizachew. Comparative feedlot performance of washera and horro sheep fed different roughage to concentrate ratio. Msc thesis, School of graduate studies, Haramaya university, Ethiopia 2012.
5. Blummel M, Makkar HPS, Becker K. *In vitro* gas production: A technique revisited. J Anim. Physiol. Anim. Nutri 1997a;77:24-34.
6. Cheeke PR. Applied Animal Nutrition Feed & feeding. 2nd (ed). Prince Hall Inc. New Jersy 1999, 525
7. Elghandour AE, Kholif AZ, Bastida DP, Martinez AZM, Salem. *In vitro* gas production of five rations of different maize silage and concentrate ratios influenced by increasing levels of chemically characterized extract of *Salix babylonica*. Turk J Vet Anim Sci 2015;39:186-194.
8. Goering HK, Vansoest PJ. Forage fibre analysis. Agriculture Research Service, USDA, Washington, D.C.USA 1970.
9. Gomes MJ, Dias-Da-Silva AA, De Azevedo JMT, Guedes CM. Response of lambs fed wheat straw based diets to supplementation with soybean hulls. Aust. J. Agric. Res., 2004;55:261-272.
10. Jaishankar N, Ramachandra B, Thirumalesh T, Ram J, Biradar U, Suranagi M. Utilization of unconventional sugarcane trash as feed in narisuwarna x kenguri sheep. International Journal of applied and pure science and agriculture, 2017;3:52-55. DOI:10.22623/IJAPSA.2017.3053.BQ6SL.
11. Krishnamoorthy U, Soller H, Steingass H, Menke KH. A comparative study on rumen fermentation of energy supplements *in vitro*. J Anim. Physiol. Anim. Nutr 1991;65: 28-35.
12. Kumar S, Dagar SS, Sirohi SK, Upadhyay RC, Puniya AK. Microbial profiles, *in vitro* gas production and dry matter digestibility based on various ratios of roughage to concentrate. Ann Microbiol. 2013;63:541-545.
13. Mahala AG, Elseed ANMF. Chemical composition and *in vitro* gas production characteristics of six fodder trees leaves and seeds. Res. J Agri. Biol. Sci 2007;3(6):983-986.
14. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Devpt 1988;28:7-55.
15. Nalini Kumari N, Ramana Reddy Y, Michel Blummel D, Nagalakshmi, Monika T, Subba Reddy BV *et al*. Growth performance and carcass characteristics of growing ram lambs fed sweet sorghum bagasse based complete rations varying in roughage to concentrate ratios. Trop Anim Health Prod 2012; 45:649-655.
16. Nalini Kumari N, Ramana Reddy Y, Bümmel M, Nagalakshmi D, Monika T, Venkata Subba Reddy B *et al*, Growth performance and carcass characteristics of growing ram lambs fed sweet sorghum bagasse based complete rations varying in roughage-to concentrate ratios. Trop. Anim. Health Prod 2013;45:649-655.
17. Ramana Reddy Y, Kumari NN, Monika T, Pavani M, Sridhar K. Evaluation of sorghum stover based complete rations with different roughage to concentrate ratio for efficient microbial biomass production by using *in vitro* gas production technique. J Anim. Res 2015;5:47-52.
18. Ramana Reddy Y, Nalini Kumari N, Monika T, Sridhar K. Evaluation of optimum roughage to concentrate ratio in maize stover based complete rations for efficient microbial biomass production using *in vitro* gas production technique, Vet. World 2016;9(6):611-615.
19. Reddy RR, Prasad DA. Evaluation of complete diets containing cowpea hay for lambs. Indian J Anim. Nutr 1983;53:206- 208.
20. Seshaiah CV, Reddy YR, Rao SJ, Srivani M. Prediction of optimum roughage to concentrate ratio in sweet sorghum (*Sorghum bicolor* L. Moench) bagasse based total mixed ration for buffaloes using *in vitro* gas technique. J Adv. Vet. Anim. Res 2014;1:224-227.
21. Venkateshwarlu V, Venkateswarlu M, Ramana Reddy Y, Harikrishna CH. Effect of feeding maize stover based complete rations on growth and carcass characteristics in Nellore x Deccani ram lambs. Indian J Anim. Nutr 2014;31:36-43.