Int J Pharm Bio Sci 2012 Oct; 3(4): (B) 247 - 257

Research Article Bio Chemistry

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

OPTIMIZATION OF ROUGHAGE TO CONCENTRATE RATIO IN SWEET SORGHUM BAGASSE BASED COMPLETE RATION FOR EFFICIENT MICROBIAL BIOMASS PRODUCTION IN SHEEP USING *IN VITRO* GAS TECHNIQUE

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ABSTRACT

An *in vitro* study was conducted to evaluate the optimum roughage to concentrate ratio in complete rations using unconventional sweet sorghum bagasse (SSB), a by product of ethanol industry as sole roughage source. Eight rations were prepared with roughage to concentrate ratio of 100:0 to 30:70. Significantly (P<0.01) higher *in vitro* gas production volume (ml) at 24 h incubation, *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) were recorded for the rations with roughage (R) to concentrate (C) ratio of 80R:20C to 30R:70C compared to 100R:0C and 90R:10C rations. Whereas, truly digestible organic matter (TDOM) was significantly (P<0.01) higher for the rations 90R:10C to 30R:70C compared to sole SSB. Among all the rations, 30R:70C has shown highest (P<0.01) IVOMD, ME and TDOM and the trend observed in ME, TDOM values reflected that, as the concentrates proportion increased, these values were also proportionately increased. The partitioning factor (PF, mg/ml) value obtained was ranged from 2.79±0.01 to 3.18±0.01 for the experimental rations. The rations from 90R:10C to 30R:70C were significantly (P<0.01) higher in PF, microbial biomass production (MBP) and efficiency of microbial biomass production (EMBP) compared to the ration contained 100 per cent SSB. There was no significant difference observed for PF and EMBP among the rations from 60R:40C to 30R:70C, wherein the SSB proportion decreased from 60 to 30 per cent in the rations. Where as for MBP, no significant difference was observed in the rations from 50R:50C to 30R:70C. These rations have also shown higher IVOMD resulting in higher microbial biomass synthesis. The less intense microbial activity was reflected by low volumes of gas produced in the rations of sole SSB to 70R:30C. The NH_3-N at 24 h of incubation was significantly (P<0.01) increased as the proportion of concentrate increased in the ration. Significantly (P<0.01) lowered total volatile fatty acid concentration was observed in the rations VII, VIII followed by V and VI. Therefore, the present study suggested that sweet sorghum bagasse (SSB) can be included in complete rations for ruminants at the level of 50-60 per cent for economic milk and meat production.

KEY WORDS: Sweet sorghum bagasse, roughage: concentrate ratio, complete ration, microbial biomass production, *in vitro* gas technique

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INTRODUCTION

Productivity of most of the Indian sheep is below their genetic potential, which is due to various factors like scarcity and fluctuation of the quality and quantity of animal feed supply throughout the year. They are mostly maintained on grazing and feeding locally available crop residues and agro-industrial by-products, which are poor in protein, energy, minerals and vitamin contents. They can make efficient use of crop residues and other non-conventional feed sources being equipped with rumen microbial ecosystem. However, this can be achieved by preparing the complete rations with agro-industrial by products and concentrate ingredients blended together. Further, the roughage to concentrate ratio in ruminant rations is one of the most important factors for efficient utilization of dietary nutrients for production. The supplementation of concentrate mixture is aimed at maximizing the ruminal microbial protein (MP) production. High MP production decreases the need for supplementing rumen undegradable feed protein by proportionally increasing carbon and nitrogen fixation into microbial cells there by reducing fermentative carbon (C) losses in CO_2 and CH_4 and nitrogen (N) losses in the urine[1].

Thus, an attempt was made to incorporate, sweet sorghum bagasse (SSB), a by-product of ethanol industry after extracting the juice from the plant, in complete rations as sole roughage source in different roughage to concentrate ratios. The *in-vitro* gas production technique was used to predict the efficiency of microbial production of SSB based complete rations with different ratios of roughage and concentrates with the objective of identifying optimum ratio for maximizing microbial production and minimizing C and N release into the environment by ruminants.

MATERIALS AND METHODS

(i) Samples preparation

Feed samples of SSB, concentrate mixture (maize, 31.0; groundnut cake, 16.5; sunflower cake, 20.0; deoiled rice bran, 23.0; molasses, 5.0; urea, 1.5; mineral mixture, 2.0 and salt,1.0 parts) with 19.16 per cent crude protein (CP) were prepared by grinding in Willey mill with 1 mm screen and 200 mg dry weight of the samples were weighed in triplicate with roughage (R) to concentrate (C) ratio of 100:0 (Ration-I), 90:10 (Ration-II), 80:20 (Ration-III), 70:30 (Ration-IV), 60:40 (Ration-V), 50:50 (Ration-VI), 40:60 (Ration-VII) and 30:70 (Ration-VIII).

(ii) In vitro gas production technique[2]

Media was prepared by using various solutions (10 ml distilled water, 0.0025 ml micro mineral solution, 5 ml bicarbonate buffer, 5 ml macro mineral solution, 0.025 ml resazurine solution and 0.06 ml reduction solution)[30]. Rumen liquor was obtained with the help of a stomach tube fitted with vacuum pump from three Deccani rams that were fed chopped SSB with supplementation of concentrates before offering the morning feed. Approximately 350 ml of rumen liquor was siphoned from different depths and directions of reticulo rumen and transferred into pre heated thermos flask, strained through a four fold muslin cloth and flushed with CO_2. Rumen fluid–buffer media mixture is prepared under continuous flushing with CO_2 and a 30 ml of inoculum consisting of 10 ml rumen fluid and 20 ml of a bicarbonate-mineral distilled water mixture was injected into the prewarmed glass syringes containing 200 mg sample and incubated in the water bath at 39^{o}C for 24 h.

Cumulative gas production was measured at 0, 3, 6, 9, 12, 15, 18, 21 and 24 h of incubation[2]. Gas production at 24 h, corrected for blank and standards, was used for determining the *in vitro* organic matter (IVOMD) digestibility[3] and metabolizable (ME) energy[2]. Partitioning factor (PF) was calculated as the ratio of substrate truly degraded : gas volume produced. The microbial biomass production (MBP) and efficiency of microbial biomass production (EMBP) of experimental rations was determined by measuring the ratio of truly digested organic matter (TDOM) and gas production[4].

(iii) Ammonia N and total volatile fatty acids estimation

After 24 hr of incubation, rumen liquor fluid–media mixture samples were analyzed for ammonia nitrogen[5] and total volatile fatty acids[6] (TVFA).

(iv) Statistical analysis

Statistical analysis of the data was carried out[7]. Least-square Analysis of variance was used to test the significance of various treatments and the difference between treatments means was tested for significance by Duncan's new multiple range and F Test[8].

RESULTS AND DISCUSSION

Chemical composition of the rations of *in vitro* gas prodction study is presented in Table 1. The CP content of the rations increased (3.94 to 14.60%) as the proportion of concentrates increased from ration I to VIII, while crude fibre (CF) content decreased (37.58 to 22.55%) from ration I to VIII as the proportion of roughage was decreased. Similar trend was observed for fibre fractions. The *in vitro* gas production at 0, 3, 6, 9, 12, 15, 18 and 24 h incubation time of different rations is presented in Table 2 and Fig. 1. The total gas produced (ml/200mg) in 24 h was increased from 42.67 in ration I to 50.17 in ration VIII. The increase in gas production was significant ($P<0.01$) among the rations as the concentrate level increased from 0 to 70 per cent, while there was no significant difference observed between ration III and IV, V and VI and VI and VII (Table 3). The decreased *in vitro* gas production volume might be due to decreased microbial fermentation in the higher roughage proportions in the rations. The higher gas production of rations containing higher proportion of concentrates might have resulted from the increased production of propionate as carbon dioxide is produced when propionate is made by ruminal bacteria via the succinate: propionate pathway. *In vitro* gas production reflects ruminal apparent substrate degradability[9] which is defined as the amount of feed fermented to short chain fatty acids and gases[10]. A decrease in gas volume was observed as the red gram straw level increased in the complete ration by replacing the concentrate proportion[11,12].

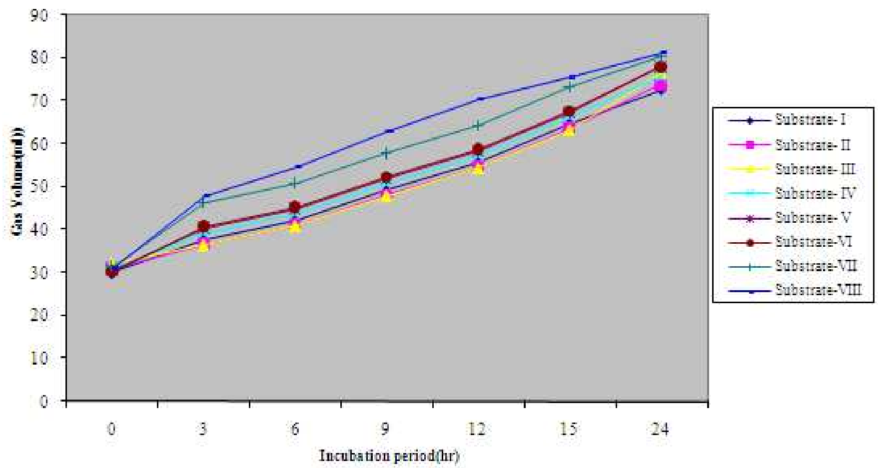

Figure 1
Effect of SSB to concentrate ratio on *in vitro* gas production

The differences in gas production for different rations was due to the suppressing effect of high cell wall and lignin present in these feeds resulting in decreased attachment of ruminal microbes to feed particles[13]. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate and gas production from protein fermentation is relatively small as compared to carbohydrate fermentation[14]. Higher gas values obtained for the experimental rations from II to VI, indicating a better nutrient availability for rumen microorganisms[15]. The findings of the present study were in agreement with the observations that, the gas production was higher (P<0.05) on high energy levels[16]. On increasing the sugarcane bagasse level in complete feed, gas production per unit digestible dry matter (DM) or organic matter (OM) was reduced[17]. Further, there was a positive correlation between CP and the rate of gas production and negative correlations between neutral detergent fibre (NDF) and acid detergent fibre (ADF), and the rate and extent of gas production[18,19].

Significantly (P<0.01) higher IVOMD for the rations III to VIII was observed compared to the ration I and II (Table 3). Among all the rations, ration VIII (70% concentrate) has shown highest (P<0.01) IVOMD. The decrease in IVOMD from ration VIII to I might be due to decrease in readily available energy, protein contents and increase in structural carbohydrates content of complete rations which might have impaired microbial growth and fermentation. An increase in the *in-vitro* organic matter degradability on high-energy rations was observed[16]. A decrease in the IVOMD in rations containing sunflower heads and maize cobs as the roughage portion increased in the complete ration was also reported[20]. The high positive correlation between gas production and OM digestibility has been reported[21]. The findings of the present study were also supported by the reports that, the roughages deficient in fermentable carbohydrates, were reflected by relatively low OM digestibility[22].

ME was significantly (P<0.01) higher for the rations III to VIII compared to ration I and II, which contained 100, 90 per cent SSB, where as, TDOM was significantly (P<0.01) higher for the rations II to VIII compared to I (Table 3). Among all the rations, ration-VIII (70% concentrate) has shown highest (P<0.01) ME and TDOM compared to other rations and the trend observed in ME, TDOM values reflected that, as the concentrates proportion increased, these values were also proportionately increased. The increased ME value was in accordance with the increased IVOMD values of rations containing higher proportion of concentrates, which reflected low digestibility of a nutrient resulted in low ME availability. The increased TDOM was a reflection of increased IVOMD values of the rations. The higher fermentable carbohydrates and available nitrogen, *i.e.* a better nutrient availability for rumen microorganisms was reported when crop residues were supplemented with concentrates[13,15,23,24]. The predictive ME values (8.13 to 9.90) were found within the range of reported values for a large number of feedstuffs[25].

The rations from II to VIII were significantly (P<0.01) higher in PF, MBP and EMBP compared to ration I, which contained 100 per cent SSB. There was no significant difference observed for PF and EMBP among the rations from V to VIII, wherein the concentrates proportion increased from 40 to 70 per cent in the rations, where as for MBP, no significant difference was observed in the rations from VI to VIII. Significantly (P<0.01) higher PF, MBP and EMBP were obtained as the proportion of concentrates increased in the rations in the present study, whereas these values were not significantly different among the rations V, VI, VII and VIII. Partitioning factor is an index of the distribution of truly degraded substrate between microbial biomass and fermentation waste products[26].When less gas is produced per unit weight of substrate truly degraded, proportionately more substrate is converted into microbial biomass, which means that, a higher PF would reflect higher conversion of truly degraded substrate into microbial biomass and *vice versa* and higher PF rations increased microbial yield[27]. In an *in vivo* study with sheep, microbial biomass flow to the duodenum was positively correlated with the PF of the mixed ration[28]. In the present study, the rations VI to VIII containing higher PF of 3.16 and 3.18 have shown higher EMBP values than the values reported for mixed rations[26].

The concentration of rumen ammonia nitrogen was significantly (P<0.01) different among the experimental rations (Table 4). The results of the present study were also in agreement with the earlier reports [29]. The increased ammonia nitrogen in the rations V to VIII could be due to active degradation of protein and hydrolysis of NPN substances in the rumen. The significantly (P<0.01) lowered total volatile fatty acid concentration was observed in the rations VII, VIII followed by V and VI, which was correlating with higher gas volume as the generated gas also comes from buffering the VFA generated from fermentation. The lowered TVFA was reflected in higher microbial biomass production as the VFA were utilized efficiently by the rumen microorganisms.

The values for PF and EMBP were similar among the V, VI, VII and VIII rations and these four rations were significantly (P<0.01) higher in ME, PF and EMBP compared to other rations. The higher EMBP was positively correlated with higher PF of the rations[27,28]. These rations have also shown higher IVOMD resulting in higher microbial biomass synthesis[4].

Table 1
Chemical composition (% DM) of experimental rations with different SSB to concentrate ratios

Parameter	Ration							
	I	II	III	IV	V	VI	VII	VIII
Proximate principles								
Organic matter	90.75	90.67	90.58	90.49	90.40	90.31	90.23	90.14
Crude protein	3.94	5.46	6.98	8.51	10.03	11.55	13.07	14.60
Ether extract	1.89	1.92	1.94	1.96	1.98	2.00	2.03	2.05
Crude fibre	37.58	35.44	33.29	31.14	28.99	26.85	24.70	22.55
Nitrogen free extract	47.34	47.85	48.37	48.88	49.40	49.91	50.43	50.94
Total ash	9.25	9.33	9.42	9.51	9.60	9.69	9.77	9.86
Cell wall constituents								
Neutral detergent fibre	74.76	70.21	65.66	61.11	56.55	52.00	47.45	42.90
Acid detergent fibre	42.93	40.24	37.55	34.86	32.17	29.48	26.79	24.10
Hemicellulose	31.84	29.97	28.11	26.25	24.39	22.52	20.66	18.80
Cellulose	37.75	34.86	31.96	29.06	26.16	23.27	20.37	17.47
Lignin	4.24	4.08	3.92	3.75	3.59	3.43	3.27	3.11

Each value is the average of triplicate analysis
Rations: I- R100: C0; II- R90:C10; III- R80: C20; IV- R70: C 30; V-R60: C40; VI-R50:C50; VII-R40:C60; VIII R30-C70

Table 2
Effect of sweet sorghum bagasse (SSB) to concentrate ratio on in vitro gas production (IVGP) volume (ml)

Ration	Incubation period (h)							SEM
	0	3	6	9	12	15	24	
I	30.00	37.67	42.17	49.17	55.67	64.67	72.67	5.72
II	31.00	36.67	41.17	48.17	54.67	63.67	74.00	5.78
III	32.00	36.33	40.83	47.83	54.33	63.33	76.67	5.99
IV	31.00	39.17	43.67	50.67	57.17	66.17	75.83	5.92
V	30.50	40.33	44.83	51.83	58.33	67.33	78.17	6.18
VI	30.17	40.67	45.17	52.17	58.67	67.67	78.00	6.19
VII	31.33	46.33	50.83	57.83	64.33	73.33	80.33	6.31
VIII	31.00	47.83	54.33	64.07	70.23	76.90	81.17	6.68
SEM	0.19	1.50	1.56	1.66	1.65	1.52	0.59	

Each value is the average of triplicate analysis
Rations: I- R100: C0; II- R90:C10; III- R80: C20; IV- R70: C 30; V-R60: C40; VI-R50:C50; VII-R40:C60; VIII R30-C70

Table 3
Effect of SSB to concentrate ratio on in vitro gas production parameters in Deccani sheep

Ration	Gas volume (ml)	IVOMD (mg)	ME (MJ/kg)	PF	TDOM (mg)	MBP (mg)	EMBP (%)
I	42.67[e]	93.87[e]	8.13[g]	2.79[d]	119.08[h]	25.21[e]	21.17[d]
II	43.00[e]	94.60[e]	8.27[g]	2.94[c]	126.60[g]	32.00[d]	25.28[c]
III	44.67[d]	98.27[d]	8.60[f]	2.94[c]	131.12[f]	32.85[d]	25.06[c]
IV	44.83[d]	98.63[d]	8.74[e]	3.06[b]	136.95[e]	38.32[c]	27.98[b]
V	47.67[c]	104.87[c]	9.21[d]	3.12[ab]	148.93[d]	44.06[b]	29.59[ab]
VI	48.17[bc]	105.97[bc]	9.37[c]	3.18[a]	153.21[c]	47.25[a]	30.84[a]
VII	49.00[b]	107.80[b]	9.61[b]	3.18[a]	155.80[b]	48.00[a]	30.81[a]
VIII	50.17[a]	110.37[a]	9.90[a]	3.16[a]	158.43[a]	48.077[a]	30.34[a]
SEM	0.57	1.24	0.13	0.04	3.39	2.27	1.15

[a, b, c, d, e, f, g, h]values bearing different superscripts in a column differ significantly (P<0.01)
Rations: I- R100: C0; II- R90:C10; III- R80: C20; IV- R70: C 30; V-R60: C40; VI-R50:C50; VII- R40:C60; VIII R30-C70

Table 4
Effect of SSB to concentrate ratio on ammonia N and TVFA concentration in rumen fluid–media mixture after 24 h incubation in Deccani sheep

Ration	Ammonia N (mg/100ml)	TVFA (meq/l)
I	14.40[g]	24.00[c]
II	16.27[fg]	33.33[ab]
III	17.87[ef]	36.0[a]
IV	19.73[e]	34.00[ab]
V	29.07[d]	31.67[b]
VI	33.07[c]	33.00[b]
VII	41.60[b]	26.00[c]
VIII	51.2[a]	23.00[c]
SEM	2.62	1.01

[a, b, c, d, e, f, g,] values bearing different superscripts in a column differ significantly (P<0.01)
Rations: I- R100: C0; II- R90:C10; III- R80: C20; IV- R70: C 30; V-R60: C40; VI-R50:C50; VII- R40:C60; VIII R30-C70

CONCLUSIONS

The present study showed that, the rations containing 60, 50, 40 and 30 per cent SSB were proved to be as best levels of inclusion along with concentrates in complete rations for growth and milk production in ruminants. However, for economic rearing of the ruminant animals, 50-60 per cent level can be considered as optimum level of sweet sorghum bagasse incorporation as sole roughage source in the complete rations of ruminant livestock.

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