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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

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ABSTRACT

An *in vitro* study was conducted to evaluate the optimum roughage to concentrate ratio in complete rations using sorghum stover (SS) as a roughage source since it is a main feed resource for urban and peri-urban dairies. Eight complete rations were prepared with roughage (R) to concentrate (C) ratio of 100R:0C to 30R:70C. *In vitro* gas production (ml) at 24 h incubation, *in vitro* organic matter digestibility and metabolizable energy, truly digestible organic matter and ammonia nitrogen production were increased linearly (P<0.01) as the proportion of concentrate was increased in the ration. Significantly higher (P<0.01) total volatile fatty acid concentration was observed in 50R:50C, 40R:60C and 30R:70C compared to other rations. Significantly (P<0.01) highest partitioning factor, microbial biomass production and efficiency of microbial biomass production were recorded at 60R:40C ratio followed by 50R:50C. Therefore, the present study suggested that SS can be included in complete rations for ruminants at the level of 60 per cent for economic milk and meat production.

Keywords: Sorghum Stover, Complete ration, *In vitro* gas technique, Roughage to concentrate ratio.

Crop residues left after harvesting grain from cereal crops are the main source of roughage for feeding of ruminants in arid and semi-arid regions of India. Currently they constitute more than 40 per cent of dry matter in Indian ruminant diets (Rao *et al.,* 2003). More recently Ramachandra *et al.* (2005) estimated that crop residues will provide more than 70% of the feed resources for the Indian ruminant population by the year 2020. However, crop residues are low in protein, energy and other important micronutrients essential for animal production. Therefore incorporation of concentrate ingredients in crop residue based diets is recommended to overcome nutrient deficiencies and optimize the efficiency of crop residues utilization in ruminants for growth, gestation and milk production. The concept of complete diet was promoted by different research workers in India where both crop residues and concentrate ingredients were processed and blended together before feeding for efficient utilization of crop residues by ruminants. It is important to remember here that the ratio of crop residues and concentrate in the ruminant diets greatly influences the economics and efficiency of utilization of dietary nutrients. 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 (Blümmel *et al.,* 1999).

Sorghum is an important staple food crop in semi-arid tropical areas and its stover is the main feed resource for urban and peri-urban dairies. Hence, in the present study, sorghum stover was used as roughage source in the complete diet and the optimum roughage to concentrate ratio was determined by using *in-vitro* gas technique. *In-vitro* gas measuring technique is one of the easy and inexpensive methods of feed evaluation, and a high correlation between gas production *in-vitro* and *in-vivo* apparent digestibility was reported (Menke and Steingass, 1988). Therefore, the objective of the above study was to determine the digestibility, gas production and ME of sorghum stover based complete diets to optimize the ratio of sorghum stover and concentrate mixture in complete diets for efficient microbial biomass production.

MATERIALS AND METHODS

Experimental diets

Sorghum stover and 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) and 70% total digestible nutrients (TDN) were ground in willey mill (Direct drive-3010-014) with 1 mm screen and prepared complete diets with roughage to concentrate ratio of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60 and 30:70.

Table 1. Chemical composition (% DM) of experimental feeds

Parameter	Sorghum stover	Concentrate mixture
Dry matter	95.4	89.5
Organic matter	89.51	89.65
Crude protien	3.79	20.52
Crude fibre	43.21	16.89
Eether extract	1.11	1.82
Nitrogen free extract	41.42	49.33
Total ash	10.49	10.35
Neutral detergent fibre	70.11	27.19
Acid detergent fibre	46.19	18.21
Hemi-cellulose	23.89	9.32
Cellulose	36.09	13.51
Lignin	10.72	3.89

Invitro gas technique

In the *in-vitro* gas technique, 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) (Menke and Steingass, 1988). Rumen liquor was obtained with the help of a stomach tube fitted with vacuum pump from three Deccani rams that were fed chopped SS 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 fourfold 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 bicarbonate mineral distilled water mixture was injected into the prewarmed glass syringes containing 200 mg sample of sorghum stover based complete diets with roughage to concentrate ratio of 100:0 to 30:70 and incubated in the water bath at (FORTUNA®, Häberle Labortechnik. Germany) 39°C for 24 h.

The gas produced was recorded at 8 and 24 hours of incubation. Gas production at 24 h, corrected for blank and standards, was used for determination of organic matter digestibility (OMD) (Krishnamoorthy *et al.*, 2005) and ME (Menke and Steingass, 1988). Partitioning factor (PF), which is calculated as the ratio of substrate truly degraded:gas volume produced (Blummel *et al.*, 1994). The microbial biomass production (MBP) and efficiency of microbial biomass production (EMBP) of experimental rations was determined using the formulae as described by Blummel *et al.* (1997). After 24 hr of incubation, rumen liquor fluid-media mixture samples were analyzed for ammonia nitrogen (Conway, 1957) and total volatile fatty acids (TVFA) (Barnett and Reid, 1956). Feed samples were analysed for proximate principles (AOAC, 1997) and fiber fractions (Van Soest *et al.* 1991).

Statistical analysis

The data was subjected to one way analysis of variance. The differences between the means were tested for significance using Duncan's multiple range test (Duncan 1955). All the statistical procedures were carried out as per the methods described by Snedecor and Cochran (1994) by programming and processing in computer. Significance was considered at $P < 0.05$ levels.

Table 2. Effect of sorghum stover to concentrate ratio on *in vitro* gas production parameters in Deccani sheep

Roughage : concentrate	Gas volume (ml/200mg)	IVOMD (mg)	ME (MJ/kg)	PF	TDOM (mg)	MBP (mg)	EMBP (%)	Ammonia N (mg/100ml)	TVFA (meq/l)
100:0	44.33^{f}	97.53^{f}	8.45^{h}	2.97^{cd}	131.81^{h}	34.27^{d}	26.00^{cd}	19.20^{h}	25.67^{d}
90:10	45.67^{e}	100.47^{e}	8.73^{g}	2.94^{d}	134.07^{g}	33.60^{d}	25.06^{d}	24.00^{g}	27.00^{cd}
80:20	46.50^{e}	102.30^{e}	8.93^{f}	3.02^{bc}	140.43^{f}	38.13^{c}	27.15^{bc}	32.00^{f}	31.00^{c}
70:30	48.33^{d}	106.33^{d}	9.28^{e}	3.08^{ab}	148.99^{e}	42.66^{ab}	28.63^{ab}	36.53^{e}	35.67^{b}
60:40	49.17^{d}	108.17^{d}	9.50^{d}	3.12^{a}	153.36^{d}	45.19^{a}	29.47^{a}	43.20^{d}	37.67^{b}
50:50	50.50^{c}	111.10^{c}	9.79^{c}	3.06^{ab}	154.69^{c}	43.59^{ab}	28.18^{ab}	54.13^{c}	42.33^{a}
40:60	52.17^{b}	114.77^{b}	10.10^{b}	3.03^{bc}	157.81^{b}	43.05^{ab}	27.28^{bc}	59.73^{b}	42.00^{a}
30:70	54.50^{a}	119.90^{a}	10.51^{a}	2.97^{cd}	161.75^{a}	41.85	25.87^{cd}	67.20^{a}	42.33^{a}
SEM	0.68	1.50	0.14	0.01	2.18	0.90	0.35	3.37	1.40

^{abcdefgh} value bearing different superscripts in a column differ significantly (P<0.01); IVOMD: *In vitro* organic matte digestibility; ME: Metabolizable energy; PF: Partitioning factor; TDOM: Total digestible organic matter; MBP: Microbial protein; EMBP: Effective microbial protein; TVFA: Total volatile fatty acids

RESULTS AND DISCUSSION

Gas volume production (ml/200mg) was increased (P<0.01) linearly as increasing the concentrate proportion in the sorghum stover based complete diet from 0 to 70 and these values were ranged from 44.33 to 54.50 (Table 2). 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 (Blummel and Orskov, 1993) which is defined as the amount of feed fermented to short chain fatty acids and gases (AFRC, 1993). A decrease in gas volume was observed as the red gram straw level increased in the complete ration by replacing the concentrate proportion (Dutta *et al.*, 2002 and Baswa Reddy, 2003). 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 (Paya *et al.*, 2007). This might be the reason for lowest gas production with sole sorghum stover incubation. 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 (Makkar *et al.*, 1995). Higher gas values obtained for the experimental rations by gradual increase in concentrate proportion, indicating a better nutrient availability for rumen microorganisms (Mahala and Elseed, 2007). 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 (Chattarjee *et al.*, 2007). On increasing the sugarcane bagasse level in complete feed, gas production per unit digestible dry matter (DM) or organic matter (OM) was reduced (Hozhabri and Singhal, 2006). 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 (Nsahlai *et al.*, 1994 and Larbi *et al.*, 1998).

Similar trend as that of gas production was also observed in *in vitro* organic matter digestibility (IVOMD) and true digestible organic matter (TDOM) (Table 2). Highest (119.90 mg) and lowest (97.53 mg) IVOMD was noticed with addition of concentrate at the level of 70 and 0 per cent, respectively. IVOMD was comparable between 70R:30C and 60R:40C. The decrease in IVOMD with reducing the concentrate level (30R:70C to 100R:0C) might be due to decrease in readily available energy, protein contents and increase in structural carbohydrates content of complete rations which might have impaired fermentation. An increase in the *in-vitro* organic matter degradability on high-energy rations was observed (Chatterjee *et al.*, 2006). 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 (Nagalakshmi *et al.*, 2005). The high positive correlation between gas production and OM digestibility has been reported (Datt and Singh, 1995). The findings of the present study were also supported by the reports that, the roughages deficient in fermentable carbohydrates reflected relatively low OM digestibility (Jayasuriya, 2002). Ahmed and El-hag (2004) stated that, excess amount of hemicellulose and cellulose acts as antinutritional factors and lower the digestibility and gas production. This might be another reason for lowest gas production and IVOMD with incubation of sorghum stover solely. Carvalho *et al.* (2005) also stated that, high cellulose and hemicellulose contents could lower the organic matter digestibility.

Among all the rations, ration contained 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 shown that, poor digestibility of a nutrients resulted in lowered ME availability. The increased TDOM was a reflection of increased IVOMD values of the rations. The higher fermentable carbohydrates and available nitrogen reflecting a better nutrient availability for rumen microorganisms was reported when crop residues were supplemented with concentrates (Blummel *et al.*, 2009 and Khanum *et al.*, 2007). The predictive ME values (8.13 to 9.90 MJ/kg DM) were found within the range of reported values for a large number of feedstuffs (Krishnamoorthy *et al.*, 1995).

Total volatile fatty acids (TVFA, meq/l) production was increased with increase in the concentrate proportion of the inoculum. TVFA production was comparable among the rations contained R:C at 50:50, 40:60 and 30:70. This result was in consistent with the findings of Getachew *et al.* (1998) who concluded that TVFA production was positively correlated ($P<0.01$) with *in vitro* gas production.

The concentration of rumen ammonia nitrogen was significantly ($P<0.01$) differed linearly among the experimental rations (Table 2). The results of the present study were also in agreement with the earlier reports (Madan Mohan *et al.*, 1997). The increased ammonia nitrogen in the rations with increasing the concentrates proportion could be due to active degradation of protein and hydrolysis of NPN substances. Ammonia liberated during incubation was incorporated into microbial protein synthesis. If the availability of N and energy are not synchronized, results in lowered microbial protein synthesis (Mc Donald *et al.,* 1988). In this study, though the ammonial nitrogen (mg/100 ml) was increased linearly from 100R:0C to 30R:70C, highest ($P<0.01$) (45.19 mg) microbial biomass production (MBP) noticed at 60R:40C and 50R:50C (43.59 mg) (Table 2). This might be due to matching supply of ammonia and fermentable carbohydrates to the bacteria.

Partitioning factor (PF) is defined as the ratio of substrate truly degraded *in vitro* (mg) to the volume of gas (ml) produced by it. PF is an index of the distribution of truly degraded substrate between microbial biomass and fermentation waste products (Thirumalesh and Krishnamoorthy, 2009). 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 (Darshan *et al.*, 2007). Hence, calculation of PF in *in vitro* study provides meaningful information for predicting the microbial biomass production in the rumen from the dry matter taken. In an *in vivo* study with sheep, microbial biomass flow to the duodenum was positively correlated with the PF of the mixed ration (Blummel *et al.*, 2003). In the present investigation, highest ($P<0.01$) PF was noticed at 60R:40C and 50R:50C. The higher EMBP observed at 60R:40C was positively correlated with higher PF of the ration. Based on the truly digestible organic matter and efficiency of microbial biomass production sorghum stover can be incorporated up to 60 percent in the complete diets of ruminants for efficient nutrient utilization and animal performance.

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