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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

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ABSTRACT

The *in vitro* gas technique was used to predict the efficiency of sweet sorghum (*Sorghum bicolor* L. Moench) bagasse (SSB) based total mixed ration comprising different ratios of roughage and concentrate, with the objective of identifying optimum ratio for feeding buffaloes. 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 were recorded for the rations with roughage (R) to concentrate (C) ratio of 60R:40C to 30R:70C as compared to other rations. Whereas, *in vitro* organic matter digestibility (IVOMD) was significantly (p<0.01) higher for the rations 70R:30C to 30R:70C as compared to other rations. Metabolisable energy (ME) and truly digestible organic matter (TDOM) were significantly (p<0.01) higher for the rations 90R:10C to 30R:70C as compared to sole SSB. The rations from 90R:10C to 30R:70C were significantly (p<0.01) higher in partitioning factor (PF), microbial biomass production (MBP) and efficiency of microbial biomass production (EMBP) as compared to sole SSB. However, the PF was not significant among rations 50R:50C, 40R:60C and 30R:70C. Therefore, the present study suggested that SSB can be included in total mixed ration of buffaloes at 50-60% for economic ruminant livestock production.

Keywords

Buffaloes, *In vitro* gas technique, Roughage-concentrate ratio, Sweet sorghum bagasse, Total mixed ration

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INTRODUCTION

Sweet sorghum (*Sorghum bicolor* L. Moench) is a water use-efficient dry land crop. Recently, the crop has been considered as an important feedstock for ethanol production (Reddy et al., 2005). The bagasse is produced after juice extraction from stalks, which is available as a waste at sweet sorghum crushing units. High fiber and low nitrogen content of Sweet sorghum bagasse (SSB) resulted in low palatability and poor nutrient utilization in ruminants. Feeding of roughages in the form of total mixed ration (TMR) improved the palatability and utilization of bulky crop residues (Nagalakshmi et al., 2006).

Optimum roughage to concentrate ratio is crucial for better rumen fermentation and availability of nutrients. *In vitro* gas production (IVGP) technique described by Menke and Steingass (1988) could be used to evaluate different ratios of SSB and concentrate to find out best roughage to concentrate ratio. However, very few reports are available in India in which *in vitro* gas production technique has been used for optimization of rations for buffaloes. The present study was aimed at prediction of optimum roughage to concentrate ratio in sweet sorghum bagasse based total mixed ration for buffaloes by using *in vitro* gas production technique.

MATERIALS AND METHODS

Sample preparation: Samples of different ratios of SSB and concentrate of 100:0 to 30:70 were prepared by grinding in Wiley mill with 1 mm screen, and 200 mg dry weight of the samples were weighed in triplicate into calibrated glass syringes (100 mL), and were incubated at 39^{o}C overnight.

Preparation of media: Media were prepared in 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).

Collection of rumen liquor: Rumen liquor was collected from the rumen of cannulated Graded Murrah buffalo bull, which was maintained on sorghum straw based complete diet containing roughage (50%) and concentrates (50%). Rumen liquor was collected at 8.00 am before offering feed and water. Sampling was done by drawing rumen contents from different portion of the rumen and filtering through a four-layered muslin cloth. The collected rumen liquor was transferred to a flask flushed with CO_2 and maintained at 37^{o}C to 39^{o}C in an insulated jug.

Measurement of *in vitro* digestibility: Gas production at 24 h was used for determining the *in vitro* organic matter digestibility (IVOMD) and metabolisable energy (ME) (Krishnamoorthy et al., 2005). 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 were determined by measuring the ratio of truly digested organic matter (TDOM) and gas production (Blummel et al., 1997).

Statistical analysis: Statistical analysis of the data was carried out by following the method described by Snedecor et al. (1994). Least-square Analysis of variance was used to test the significance of various treatments, and the difference between treatments means was tested for significance study by Duncan's new multiple range and F Test (Duncan, 1955).

RESULTS AND DISCUSSION

The crude protein (CP) content of the experimental rations was found to be increased from 3.94 to 14.60%. On the other hand, crude fiber (CF) content of the rations was decreased from 37.58 to 22.55% as the proportion of concentrates increased from 0 to 70%. These findings were same to that of the report of Kumari et al. (2012).

In this study, total gas production (mL/200 mg) in 24 h was increased from 43.17 (in ration I) to 50.67 (in ration VIII) (**Table 1**). The IVGP was significantly (p<0.01) increased as the proportion of concentrate increased from 0 to 70% in the SSB based total mixed ration except between rations I and II; II and III, and III and IV (**Table 1**). Gas production is basically the result of fermentation of carbohydrates to volatile fatty acids *i.e.*, acetate, propionate and butyrate, and gas production from protein fermentation is relatively lesser as compared to carbohydrate fermentation (Makkar et al., 1995). Higher gas values obtained for the experimental rations from V to VIII, indicating a better nutrient availability to rumen microorganisms (Ahmed and Abdel, 2007). The higher gas production of rations containing higher proportion of concentrate might be due to the increased production of propionate as CO_2 is produced when propionate is made by ruminal bacteria via the succinate: propionate pathway. *In vitro* gas production reflects ruminal apparent substrate degradability. The decreased gas production in TMR having more roughage content was due to the suppersing 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). On increasing the sugarcane bagasse level in complete feed, gas production per unit digestible dry matter (DM) or organic matter (OM) was reduced (Hozhabri et al., 2006).

In our study, higher (p<0.01) IVOMD was observed from the ration IV to VIII as compared to the ration I, II and III, in which the difference was not significant (**Table 1**). The increase in IVOMD from ration IV to VIII might be due to increase in readily available energy and protein contents of the total mixed rations which might have improved microbial growth and fermentation (Chatterjee et al., 2006). Increase in the roughage component of the total mixed ration

Table 1: *In vitro* gas production (IVGP), *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME) and truly digestible organic matter (TDOM) of rations with different ratios of SSB and concentrate.

Ration	IVGP (mL/200 mg DM)	IVOMD (mg)	ME (MJ/kg DM)	TDOM (mg)
I	$43.17^{g}\pm0.17$	$94.97^{f}\pm0.37$	$8.30^{h}\pm0.02$	$122.00^{h}\pm0.01$
II	$44.17^{fg}\pm0.17$	$97.17^{f}\pm0.37$	$8.52^{g}\pm0.02$	$132.50^{g}\pm0.02$
III	$45.50^{ef}\pm0.58$	$100.10^{f}\pm1.27$	$8.79^{f}\pm0.08$	$140.30^{f}\pm0.00$
IV	$46.67^{e}\pm0.50$	$102.30^{e}\pm1.10$	$9.01^{e}\pm0.07$	$148.00^{e}\pm0.00$
V	$47.83^{d}\pm0.44$	$105.60^{d}\pm1.10$	$9.30^{d}\pm0.07$	$152.50^{d}\pm0.00$
VI	$48.33^{c}\pm0.16$	$108.90^{c}\pm1.27$	$9.59^{c}\pm0.08$	$155.60^{c}\pm0.00$
VII	$49.83^{b}\pm0.60$	$113.30^{b}\pm1.10$	$9.95^{b}\pm0.07$	$160.70^{b}\pm0.00$
VIII	$50.67^{a}\pm0.44$	$116.60^{a}\pm1.10$	$10.24^{a}\pm0.07$	$163.90^{a}\pm0.00$

[a,b,c,d,e,f,g,h] values bearing different superscripts in a column differed significantly (p<0.01)

Table 2: Partitioning factor (PF), microbial biomass production (MBP) and efficiency of microbial biomass production (EMBP) of complete rations with different ratios of SSB and concentrate.

Ration	PF (mg/mL)	MBP (mg)	EMBP (mg)
I	$2.83^{d}\pm0.01$	$27.03^{c}\pm0.37$	$22.16^{c}\pm0.30$
II	$3.00^{c}\pm0.01$	$35.33^{b}\pm0.37$	$26.67^{b}\pm0.28$
III	$3.08^{c}\pm0.04$	$40.20^{b}\pm1.27$	$28.65^{b}\pm0.91$
IV	$3.17^{b}\pm0.03$	$42.70^{a}\pm0.67$	$29.45^{a}\pm0.54$
V	$3.19^{b}\pm0.03$	$45.57^{a}\pm0.98$	$30.14^{a}\pm0.66$
VI	$3.22^{a}\pm0.01$	$47.30^{a}\pm1.30$	$30.28^{a}\pm0.82$
VII	$3.23^{a}\pm0.03$	$49.37^{a}\pm1.02$	$30.35^{a}\pm0.64$
VIII	$3.24^{a}\pm0.03$	$50.90^{a}\pm1.01$	$30.39^{a}\pm0.66$

[a,b,c,d] values bearing different superscripts in a column differed significantly (p<0.01)

decreased the IVOMD (Nagalakshmi et al., 2005), and OM digestibility (Jayasuriya, 2000).

ME and TDOM were significantly (p<0.01) increased in all the rations from II to VIII as the proportion of concentrate increased (**Table 1**). 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 (Ahmed et al., 2007). The predicted ME values were found within the range of reported values (8.13 to 9.90) for a large number of feedstuffs (Krishnamoorthy et al., 1995).

The PF was significantly (p<0.01) differred between I and II, III and IV and V and with other rations, and the difference was not significant among the rations II and III, IV and V, and between VI, VII and VIII (**Table 2**). From the ration I to VIII, the PF increased as the level of concentrate increased in the ration. PF is an index of the distribution of truly degraded substrate between microbial biomass and fermentation waste products, as reported by 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.

There was no significant difference found in MBP and EMBP (mg) among different rations except between I and II, and between I, II, III and other rations in which the difference was significant (p<0.01) (**Table 2**). The EMBP (mg) values increased with increasing concentrate in the rations from I to VIII rations. The higher MBP and EMBP were positively correlated with higher PF of the rations (Darshan et al., 2007).

CONCLUSIONS

Optimum proportion of sweet sorghum bagasse incorporation in total mixed ration is important for efficient utilization of fibrous diet in buffaloes. Based on the results obtained in the present study, rations V and VI having roughage to concentrate ratio of 50:50 and 60:40% level can be considered as optimum level for preparation of SSB based TMR to feed buffaloes economically.

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