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Vishal SB
PG Scholar, Department of
Animal Nutrition, Veterinary
College, Hassan, Karnataka,
India

Jaishankar N
Professor, Department of
Animal Nutrition, Veterinary
College, Bengaluru,
Karnataka, India

Thirumalesh T
Professor and Head,
Department of Animal
Nutrition, Veterinary College,
Shivamogga, Karnataka, India

Guruprasad R
Associate Professor,
Department of Livestock
Production Management,
Veterinary College, Hassan,
Karnataka, India

Vinuthan MK
Associate Professor,
Department of Veterinary
Physiology and Biochemistry,
Veterinary College, Hassan,
Karnataka, India

Harish Shenoy
Associate Professor, KVK,
Mangalore, KVAFSU, Bidar,
Karnataka, India

Roopa TK
Assistant Professor,
Department of Livestock Farm
Complex, Veterinary College,
Hassan, Karnataka, India

Corresponding Author:
Vishal SB
PG Scholar, Department of
Animal Nutrition, Veterinary
College, Hassan, Karnataka,
India

In vitro rumen fermentation kinetics and microbial biomass synthesis of some non-leguminous annual fodder crops using cattle inoculums

Vishal SB, Jaishankar N, Thirumalesh T, Guruprasad R, Vinuthan MK, Harish Shenoy and Roopa TK

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Abstract

The samples of non-leguminous annual fodder crops *i.e* Maize, Sorghum, Bajra and Ragi obtained after first cut were subjected for chemical analysis, fibre fractions and evaluated for rumen *in vitro* gas production kinetics and microbial biomass synthesis. The fodder Bajra recorded minimum metabolizable energy (ME, MJ/kg DM) content and Gas production over 24hr (ml/g DM) among all fodders. The $t_{1/2}$ observed for Maize and Sorghum was 15 (h) whereas, for Bajra and Ragi it was around 12 (h) and rate of gas production (k, h^{-1}) was around 5 percent per hour in almost all non-leguminous annual fodders. The potential gas production was also lower in Bajra when compared with other non-leguminous fodder crops. The true digestible organic matter (mg/g DM) was significantly higher in Sorghum followed by Maize, Ragi and Bajra. PF value of Maize was lower than the PF value of other non-leguminous annual fodders. Therefore, MBP and EMBS values were also lower for Maize when compared with other non-leguminous annual fodder crops. However significantly ($P < 0.01$) higher MBP (mg) was noticed in Ragi and EMBS was also higher in Ragi when compared with Bajra, Sorghum and Maize. As energy values and CP are comparable, based on microbial biomass production Ragi and Bajra among non-leguminous fodder crops can be recommended to include in the diets of ruminants for higher production microbial protein to meet the requirements of Total protein for growth and milk production.

Keywords: Ragi, Bajra, maize, sorghum, microbial biomass production

Introduction

India's agricultural production system is heavily reliant on livestock rearing, which plays a critical role in the socioeconomic development of millions of rural households and contributes significantly to the nation's economy. According to the 2019 census, India's livestock population stood at 535.82 million, reflecting a 4.6% increase since the 2012 census. Despite this growth, the Indian livestock sector faces substantial feed shortages, including a 44% deficit in concentrate feed ingredients, an 11% deficit in dry fodder, and a 35.6% deficit in green fodder (IGFRI Vision, 2050). Singh *et al.* (2022) [28] projected that by 2050, the demand for dry fodder will reach 631 million tonnes (MT) and for green fodder 1,012 MT. If production trends continue at the current rate, the deficit in dry and green fodder is expected to be 13.2% and 18.4%, respectively. This gap can be mitigated by expanding the area under fodder cultivation, improving yields through modern cultivation practices, and using high-quality seeds and planting materials. The utilization of crop residues, such as unpalatable sugarcane trash, also presents a viable solution. Urea-ammoniated sugarcane trash has been shown to have a nutritional composition comparable to other crop residues, with a voluntary intake of 2% of body weight, highlighting its potential as a valuable forage resource (Jaishankar *et al.*, 2021) [15, 16]. While non-leguminous fodder crops generally produce higher dry matter yields per hectare due to their carbohydrate-rich composition, taller growth habit, and greater biomass production, their nutritional quality may be lower compared to legumes. In this context, several non-leguminous fodder crops were cultivated with the objectives of evaluating their proximate composition, fiber fractions, metabolizable energy, rumen fermentation kinetics, and microbial biomass synthesis in the rumen.

Materials and Methods

The experiment was conducted in the fodder museum maintained by department of Livestock Farm Complex, Veterinary College, Hassan. Somenon-leguminous annualfodder crops were cultivated and were harvested manually at right stage to utilise as green fodder. After harvesting, the fodder crops were sampled and dried at 60 °C in a forced air oven till constant weight and ground through 1 mm sieve using a Willey mill. Airtight containers were used to store the milled samples, till further analysis. The representative samples of some non-leguminous annual fodders i.eMaize, Sorghum, Bajra and Ragi were examined for fiber fraction and proximate principles, and after that, the samples underwent rumen *in vitro* gas production method (RIVGPT) in accordance with the protocol outlined by Menke and Steingass (1988) [22] to calculate the fodder's metabolizable energy content. To determine the kinetics of gas production, nonlinear regression was used to predict the rate and volume of gas production based on the cumulative gas production at incubation times varying from 2 to 96 hours.

Rumen fluid was collected from healthy cattle weighing 200 kgs aged 5 years, using stomach tube and vacuum pump. In order to obtain a thorough understanding of the fermentation pattern, the samples were evaluated *in vitro* using the techniques described by Menke and Steingass (1988) [22]. The ME content of selected non leguminous annual fodder crops was calculated according to the procedure described by Menke and Steingass (1988) [22], using rumen *in vitro* gas production technique (RIVGPT). Rumen fermentation kinetics using the model $Y = a + b(1 - e^{-kt})$, rate and extent of gas production was calculated. Where Y represents gas volume (ml) at time t, 'a' is the gas produced by soluble fraction, 'b' is gas produced by fraction that is insoluble but degradable, D (a + b) is potential gas production (ml), and k is the rate (per hour) at which gas is generated (Krishnamoorthy *et al.*, 1991) [20], $\ln 2/k$ was used to calculate the time at half asymptotic gas was produced ($t_{1/2}$). The microbial biomass synthesis (MBS) of some non-leguminous fodder crops was determined by finding the ratio of TDOM (total digestible organic matter), gas production at the moment of half maximal gas production ($t_{1/2}$), and Partitioning factor (PF) as described by Blummel, *et al.* (1997) [5].

Results and Discussion

In vitro gas production

Gas production (ml/200 mg DM) data at various incubation period intervals obtained from RIVGPT are provided in [Table 2] and are graphically depicted in [Fig-1].Over the course of the incubation period, the total production of gases increased. Out of all the fodders examined, Bajra has lower net gas production, than other three of fodders. This may be due toitslower digestibility compared to other fodders.

Predicted metabolisable energy (ME) by RIVGPT

Among non-leguminous annual fodders tested for ME values by RIVGPT, significantly ($P < 0.01$) higher gas production at 24h (ml/g DM) and ME (MJ/kg) were noticed. As the gas production increased ME values also increased in non-leguminous fodder crops, which might be due to partitioning of OM digested in to higher VFA production and lead to higher energy values (Getachew *et al.*, 2004) [11]. The fodder Bajra which recorded minimum ME (MJ/kg

DM) content and GP-24hr (ml/g DM) among all fodders, which might be attributed to its comparatively higher NDF and ADF values observed than those of other fodders and also to lower rate of degradability. According to Sallam *et al.* (2008) [26], feed fermentation and the carbohydrate fraction are directly related to the gas produced during the feed's incubation in the buffered rumen fluid and so, the reduced fiber fraction composition may be related to the increased gas output. This is consistent with the findings of De Boever *et al.* (2005) [9], who found a negative correlation between gas production and NDF levels and a positive correlation with starch.

Gas production-24hr (ml/g DM) for Maize recorded in the present study can be compared to value reported by Opsi *et al.*, (2013) [24]. GP-24hr (ml/g DM) of Sorghum observed in the present study is 14.4% higher than the gas value (213.5) reported by Colombo *et al.* (2007) [7], this could be caused by variations in the animal's physiological condition, its nutrition, or its rumen fluid sources, such as animal species (Bonsi *et al.*, 1995; Getachew *et al.*, 2002) [6, 10]. Predicted ME (MJ/kg DM) value in the present study for Sorghum and Bajra are in agreement with findings of study conducted by Khanum *et al.* (2007) and Kumar *et al.* (2015) [21] respectively. Since fodder plot of Ragi in the present study, is surrounded with the plots of leguminous fodder, its Predicted ME (MJ/kg DM) is comparable to value reported by Keba *et al.* (2022) [18] for Finger millet straw when intercropped with *Vicia sativa* (leguminous plant). When compared to solo grass, the fodder quality can be enhanced by the mixed cultivation of grasses and legumes in terms of the CP content Keba *et al.* (2022) [18]. The study conducted by Karabulut (2007) [17] found a positive association between the CP concentration and gas production, as well as the ME content.

Gas production kinetics

The $t_{1/2}$ observed for Maize and Sorghum was 15 (h) whereas, for Bajra and Ragi it was around 12 (h) and rate of gas production (k , h^{-1}) was around 5 percent per hour in almost all non-leguminous annual fodder crops which indicated that the rate of fermentation is similar in cereal fodder crops studied in this experiment. The gas produced from fraction, that is easily soluble was very low in Bajra than other cereal fodder crops which may be due to presence of tannins. However, Maize, Sorghum and Ragi had similar fraction which is insoluble but degradable but higher than that of Bajra. The potential gas production was also lower in Bajra when compared with other cereal fodder crops.

The gas production kineticsi.e $t_{1/2}$ (h), k (h^{-1}), D ml of Maize and Sorghum recorded in the present study can be compared with reported values for respective fodder in the earlier study conducted by Bhatta *et al.*, (2017) [2] but for Maize PGP production (ml/g DM) is 24% higher, this could be caused by variations in the animal's physiological condition, its nutrition, or its rumen fluid sources, such as animal species (Bonsi *et al.*, 1995; Getachew *et al.*, 2002) [6, 10]. According to research by Bonsi *et al.*, (1995) [6], rumen fluid from animals fed various diets produced varying gas values at different stages of incubation. The study examined the impact of donor animal diet on *in vitro* gas generation. Trei *et al.*, (1970) [31] also noted variations in gas production between the rumen fluids of two animals who were given the identical diet.According to Getachew (2002) [10], the donor animal's diet had a significant impact on the

concentrations of bacteria, which in turn affected the *in vitro* gas production.

The k (h^{-1}), D (ml/g) of Ragi observed in the present study can be compared with values reported by Kiran and Krishnamoorthy (2007) [20] for GPU-45 variety of finger millet straw, with slightly higher (15%) D (ml/g) value. When the $t_{1/2}$ (h), k (h^{-1}) and D (ml/g) of Bajra observed in the present study compared with those of Bajra straw (15.7, 0.040, 170) reported by Bhatta *et al.*, (2017) [2], comparatively higher D (ml/g) observed in the present study for Bajra can be attributed to its higher rate of degradation and lower half-life compared to those of Bajra straw and Harvesting fodder at vegetative stage. Murugan *et al.*, (2016) [27] also stated that, Cell wall content as NDF and ADF rises in tandem with the bajra fodder's maturity at harvest. De Boever *et al.*, (2005) [9] similarly found a negative correlation between NDF levels and gas production.

Substrate degradation (TDOM) and Microbial biomass synthesis indices

The true digestible organic matter (mg/g DM) was significantly higher in Sorghum (487.13) followed by Maize (470.32), Ragi (419.61) and Bajra (387.7). The TDOM values at $t_{1/2}$ were higher for concentrate feed mixture than Succulent fodder followed by dry roughage. The lower content of hemicellulose, cellulose, and lignin show higher TDOM (Jaishankar N., *et al.*, 2018) [14]. As the gas production increase, the TDOM of non-leguminous annual fodders was also increased. Because of variation in gas production depending upon organic matter digestibility, PF value of Maize was 2.99 which is lower than PF value of other non-leguminous annual fodder crops. Therefore, MBP and EMBS values were also lower for Maize when compared with other non-leguminous annual fodders. However significantly ($P < 0.01$) higher MBP (mg) was noticed in Ragi 142.93 and EMBS was also higher in Ragi when compared with Bajra, Sorghum and Maize.

The IVDMD (%) of Maize, Sorghum, Bajra and Ragi observed in the present study are comparable to findings of earlier studies (Singh *et al.*, 1977; Gupta *et al.*, 2004; Kumar *et al.*, 2015; Negesse *et al.*, 2018) [29, 12, 21, 23]. TDOM (mg/g DM) at $t_{1/2}$ (h) for Maize, Sorghum, Bajra and Ragi were comparable to TDOM (mg/g DM) values reported Vaswani *et al.* (2016) [32], Tesfaye *et al.* (2021) [30], Salama *et al.* (2020) [25] and Backiyalakshmi *et al.* (2021) [1] respectively. PF at $t_{1/2}$ for Maize, Sorghum and Bajra is comparable with value reported by Kumar *et al.* (2015) [21]. PF at $t_{1/2}$ for Ragi observed in the present study is comparable with value reported by Darshan *et al.* (2007) [8] for Finger millet straw. Theoretically, PF of feedstuffs range from 2.75 to 4.41, representing ^YATP of 10 to 40 (BIÜmmel *et al.*, 1997) [5]. PF above 4.41 is therefore probably an analytical artifact. Higher PF is caused by any factor that either increases TDOM (soluble unfermented OM) or decreases gas production (degraded protein) (BIÜmmel *et al.*, 2003) [4]. There may be as much scope for incorporating PF, in diet formulation as there is for the amount of CP and ME in feedstuffs that are now in use (Kiran and Krishnamoorthy 2007) [8]. The PF, MBP and EMBS at $t_{1/2}$ for complete diets linearly increased with gradual increase in the proportion of concentrated feed mixture from 20 to 60% in the diet with sugarcane trash as sole roughage source, indicate easily digestible nutrients improve PF, MBP and EMBS (Jaishankar *et al.*, 2021) [15, 16].

EMBS (g/kg TDOM) values observed in the present study for leguminous and non-leguminous fodders are comparable with the range value of 160-490, with mean value 303 as reported by Van Soest, 1994 for forages. Since it was shown that the feed ingredient's PF serves as an EMBS index (BIÜmmel *et al.*, 1997) [5] and aiming for higher EMBS *in vivo* would be part of the diet design to attain higher PF (BIÜmmel and Lebzien, 2001; BIÜmmel *et al.*, 2003) [3, 4].

Table 1: Chemical composition (% on DMB) of some non-leguminous annual fodder crops

Composition	Maize	Sorghum	Bajra	Ragi
Dry matter	25.59	24.74	18.72	16.91
Organic matter	91.50	91.61	88.21	87.09
Crude protein	9.51	7.12	8.58	9.14
Ether extract	3.67	1.03	1.71	2.02
Crude fibre	22.39	25.73	22.74	23.95
Nitrogen free extractives	28.18	27.52	23.85	23.59
Tota ash	8.50	8.39	11.79	12.91
Neutral detergent fibre	62.13	60.6	67.36	66.82
Acid detergent fibre	38.46	39.21	42.91	34.62
Acid detergent lignin	4.9	3.37	5.05	3.69
Gas production-24 (ml/g DM)	242.9	244.3	169.2	238.7
Metabolizable energy (MJ/kg DM)	9.35	9.39	7.35	9.24

Table 2: The average net gas generation (ml/200 mg DM) by RIVGPT at various time intervals (h)

	Maize	Sorghum	Bajra	Ragi
2	12.7	13.0	6.3	11.0
4	17.4	17.8	7.9	15.6
6	21.2	22.0	12.9	21.0
8	25.2	25.9	17.7	26.7
10	30.2	29.8	21.5	31.2
12	35.2	33.7	25.2	35.8
16	40.3	38.9	28.0	40.6
20	43.5	43.9	30.9	44.2
24	46.8	48.8	33.8	47.9
30	50.9	51.2	36.7	51.2
36	54.9	53.5	39.7	54.5
48	59.8	60.1	42.6	58.5
60	63.5	62.9	44.2	61.1
72	65.7	65.2	45.4	62.6
96	67.2	67.4	46.3	64.1

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 $t_{1/2}$), microbial biomass production (MBP, mg) and efficiency of microbial biomass synthesis (EMBS, g/kg TDOM) for some non-leguminous fodder crops

Fodder crop	Maize	Sorghum	Bajra	Ragi
Kinetic parameters				
$t_{1/2}$ (h)	15.15	15.35	12.11	12.14
k (h^{-1})	0.046	0.045	0.057	0.057
a	38.86	41.33	3.21	24.43
b	297.44	294.63	225.74	290.93
D ml	336.3	335.95	228.95	315.35
Gas at $t_{1/2}$	154.48	161.43	114.32	124.29
Substrate degradation (mg/g DM) at $t_{1/2}$				
TDOM	470.3	487.1	387.7	419.6
IVDMD	53.97	55.98	49.14	53.66
Microbial biomass synthesis indices at $t_{1/2}$				
PF	2.99	3.03	3.35	3.34
MBP	123.7	133.3	133.2	142.9
EMBS	262.5	273.5	343.3	340.6

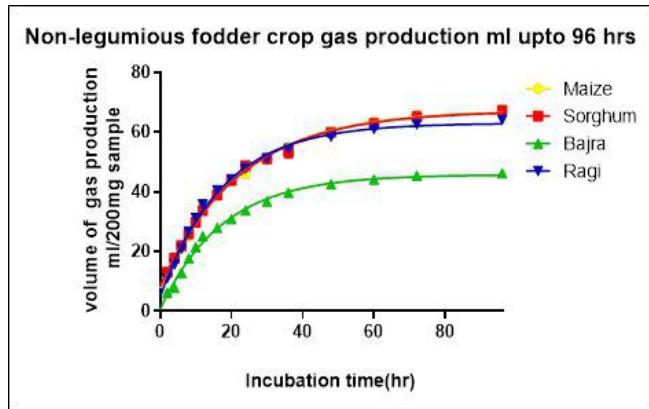


Fig 1: Rate of gas production (k) of non-leguminous annual fodder crops

Summary and Conclusion

The metabolizable energy (ME) values of the non-leguminous fodder crops, as determined by the rumen *in vitro* gas production technique (RIVGPT), ranged between 7 and 8 MJ/kg DM, with no marked variation observed among the different fodders. The rate of fermentation in these non-leguminous fodders was approximately 5% per hour, indicating a comparatively slower rate of ruminal degradation than that observed in leguminous fodders. An increase in partitioning factor (PF) values was associated with a corresponding increase in microbial biomass production, reflecting improved efficiency of substrate utilization for microbial growth. Although the energy values and crude protein (CP) contents were broadly comparable among the non-leguminous fodders, ragi and bajra exhibited relatively higher microbial biomass production. Therefore, based on their superior capacity to support microbial protein synthesis, ragi and bajra can be recommended for inclusion in ruminant diets to enhance microbial protein yield, thereby contributing to the total protein requirements for growth and milk production.

Application of research

Creation of database for at least commonly used non-leguminous annual fodder crops for their ME values, fermentation characteristics and microbial biomass synthesis indices using *in vitro* gas production technique will go a long way in using their data at least for the animal nutritionist of Karnataka in particular and India in general to formulate the rations for ruminants for improved fermentation pattern so as to improve the production and to reduce the cost of production.

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