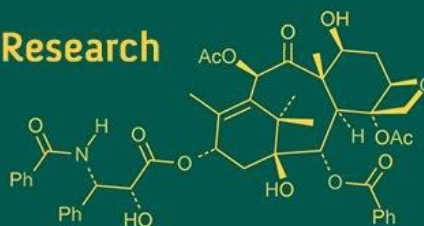


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Rumen fermentation kinetics and microbial biomass synthesis of some leguminous fodder crops by *in vitro* gas production technique

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Abstract

The samples of leguminous annual fodder crops *i.e.* Horsegram, Field bean, Cluster bean and Cowpea 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 *in vitro* gas production was gradual and maximum in Horsegram than the other fodder crops. As the gas production increased, ME values also increased. The $t_{1/2}$ (h) observed for Horsegram was 11.66 which was higher than that of Field bean, Cluster bean (8.20, 8.58) and Cowpea (7.57). As $t_{1/2}$ (h) value increased, gas production at $t_{1/2}$ (h) also increased. The rate of gas production (k , h^{-1}) in Cowpea was 9.2 per cent per hour (0.092) followed by Field bean and Cluster bean (8.1 and 8.5 per cent per hour) and Horsegram (5.9 per cent per hour) The true digestible organic matter (TDOM) of Horsegram was significantly ($P < 0.01$) higher than other leguminous fodder crops. The lowest TDOM was observed in Cluster bean. The MBP (microbial biomass production) and efficiency of microbial biomass synthesis (EMBS) was significantly higher in Horsegram which had higher partitioning factor (PF) value. Similarly, as the PF value increased the MBP and EMBS were also increased in leguminous fodders where lowest MBP was noticed in Cluster bean. As energy values and CP are comparable, based on MBP, Horsegram and Field bean among leguminous 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: Horsegram, Field bean, Cluster bean, Cowpea, *in vitro* gas production, rumen fermentation

Introduction

In the Indian livestock feeding system, cattle and buffaloes are commonly provided with green forages, silage, hay, and dry fodders. However, only a limited number of farmers supplement these with additional feeds (Shashikala *et al.*, 2017) [17]. Crop residues such as sugarcane trash have been shown to possess a nutritional composition comparable to other common crop residues. Urea-ammoniated sugarcane trash demonstrated a voluntary intake of 2% of body weight, highlighting its potential as a valuable forage resource (Jaishankar *et al.*, 2021) [11]. Leguminous fodder crops offer another important option as a protein-rich feed, with most containing approximately 16–18% crude protein (CP). Increasing the production of high-quality fodder is critical, as Indian ruminants primarily rely on low-quality roughages that are deficient in protein, energy, minerals, and vitamins. To improve the utilization of crop residues, supplying nitrogen to rumen microbes—either through cereals or leguminous fodder—is essential, especially in the context of rising concentrate feed costs. Understanding the nutritional quality of leguminous fodder crops is crucial for optimizing livestock performance. Knowledge of nutrient composition, digestibility, and predicted energy levels enables the formulation of balanced rations that support economical and efficient production (Devendra *et al.*, 2011) [8]. In this context, the present study aimed to evaluate the kinetics of *in vitro* gas production, metabolizable energy, digestibility of organic matter, and microbial protein synthesis of selected annual leguminous fodder crops. *In vitro* techniques offer a cost-effective, time-efficient alternative to *in vivo* studies, providing greater control over experimental conditions. Microbial protein synthesized in the rumen by microorganisms

constitutes the primary protein source for ruminants, making the estimation of microbial protein production efficiency a key component of ruminant nutrition.

Materials and Methods

The experiment was conducted at the Fodder Museum maintained by the Department of Livestock Farm Complex, Veterinary College, Hassan. Selected leguminous annual fodder crops were cultivated and harvested manually at the appropriate stage of growth for use as green fodder. After harvesting, representative samples of the fodder crops were collected and dried in a forced-air oven at 60 °C until a constant weight was achieved. The dried samples were then ground using a Willey mill to pass through a 1 mm sieve and stored in airtight containers until further analysis. Representative samples of selected leguminous annual fodders, namely horsegram, field bean, cluster bean, and cowpea, were analyzed for proximate principles and fiber fractions using standard procedures. To estimate the metabolizable energy content, the samples were subjected to the rumen *in vitro* gas production technique (RIVGPT) following the methodology described by Menke and Steingass (1988) [16]. Cumulative gas production was recorded at regular incubation intervals ranging from 2 to 96 hours. The kinetics of gas production, including rate and extent, were estimated using nonlinear regression models fitted to the gas production data.

The ME content of some leguminous annual fodder crops was determined by rumen *in vitro* gas production technique (RIVGPT) according to procedure described by Menke and Steingass, (1988) [16]. The kinetics of gas production, the samples were assessed *in vitro* using the methods outlined by Menke and Steingass (1988) [16] in order to completely understand the fermentation pattern. The rate and extent of gas production were computed using the model $Y = a + b(1 - e^{-k \cdot t})$. Where gas volume (ml) at time t is represented by Y , Gas generated by the soluble portion can be described as 'a'. 'b' is the gas that the insoluble but degradable component produces, Potential gas production (ml) is represented by $D(a+b)$, while the rate of gas generation (hourly) is represented by 'k' (Krishnamoorthy *et al.*, 1991) [13]. $\ln 2/k$ was used to calculate the time at half asymptotic gas was produced ($t_{1/2}$). Microbial biomass synthesis and Partitioning factor (PF) was calculated as per the method described by Blümmel *et al.* (1997) [6].

Results and discussion

In vitro gas production

Data on gas generation (ml/200 mg DM) at different periods of incubation, acquired from RIVGPT, are given in [Table 2] and illustrated graphically in [Fig-1]. The *in vitro* gas production was gradual and maximum in Horsegram than the other three fodder crops, this may be due to its higher digestibility.

Predicted metabolisable energy (ME) by RIVGPT:

Among leguminous fodder crops 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 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) [9].

Comparatively higher predicted ME (MJ/kg DM) value for Field bean and Cowpea than the value reported by Asefa (2017) and Kumar *et al.* (2015) [14] respectively, can be attributed to their high CP (%) and also to their higher EE (%) respectively. Predicted ME (MJ/kg DM) value in the present study for Cluster bean is in agreement with the value of 8.19 reported for Datt *et al.* (2009) [7]. Predicted ME (MJ/kg DM) value for Horsegram is comparable to value of 9.09 reported by Karabulut *et al.* (2007) [12] for Chick pea belonging to Fabaceae family. The study by Karabulut (2007) [12] reports a negative association between gas production and the NDF or ADF contents of legume hays and a positive correlation between the CP content and gas production.

Gas production kinetics

The gas production kinetics studied *in vitro* for leguminous annual fodder crops which is represented in the form of graph showed that, the $t_{1/2}$ (h) observed for Horsegram was 11.66 which was higher than that of Field bean, Cluster bean (8.20, 8.58) and Cowpea (7.57). It was also observed that, as $t_{1/2}$ (h) value increased, gas production at $t_{1/2}$ (h) also increased which is because of increased duration of incubation and degradability. However, the rate of gas production (k , h^{-1}) in Cowpea was 9.2 per cent per hour (0.092) followed by Field bean and Cluster bean (8.1 and 8.5 per cent per hour) and Horsegram (5.9 per cent per hour) indicated that, the rate of degradability higher in Cowpea but gas produced from easily soluble fraction was 48.07 (ml) in Field bean, 43.33 (ml) in Horsegram, 30.47 (ml) in Cowpea and 21.48 (ml) in Cluster bean which showed that, Field bean had more of soluble fraction when compared to other leguminous annual fodders. The gas produced from insoluble but degradable fraction in Horsegram 265.5 (ml), in Field bean 208.73 (ml), in Cluster bean 224.2 (ml) and in Cowpea 222.1. The potential gas production (D , ml) which was calculated by adding a and b factors was higher in Horsegram than other leguminous annual fodders. This clearly showed that, Horsegram had more of degradable organic matter than any other leguminous annual fodders studied. The lower degradability observed in the form of lower potential gas production in other leguminous fodders except Horsegram might be due to binding of organic matter to any of anti-nutrition factors.

The $t_{1/2}$ (h) and k (h^{-1}) for Horsegram observed in the present study can be compared with values reported by Bhatta *et al.*, (2017) [3] for Lucerne fodder which belongs to same family (Fabaceae) as that of Horsegram. The $t_{1/2}$ (h) and k (h^{-1}) for Cowpea observed in the present study similar to values reported by Ansah *et al.*, (2021) [1].

Table 1: Chemical composition of leguminous annual fodder crops

Composition	Horsegram	Field bean	Cluster bean	Cowpea
Dry matter	19.57	16.85	21.73	16.30
Organic matter	91.5	91.61	88.21	87.09
Crude protein	18.44	17.58	16.61	23.40
Ether extract	2.60	3.19	1.38	3.98
Crude fibre	22.39	22.74	23.95	25.73
Nitrogen free extractives	27.97	26.96	27.43	25.52
Tota ash	8.92	9.88	11.77	10.96
Neutral detergent fibre	46.03	53.67	43.92	49.90
Acid detergent fibre	44.87	45.33	36.32	41.28
Acid detergent lignin	8.72	8.37	9.19	8.68
Gas production-24 (ml/g DM)	231.9	221.2	209.1	219.7
Metabolizable energy (MJ/kg DM)	9.05	8.76	8.43	8.72

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

	Horsegram	Field Bean	Cluster Bean	Cowpea
2	12.5	13.2	9.7	12.2
4	17.5	22.1	15.5	18.4
6	25.5	28.6	22.5	26.9
8	31.6	31.7	27.8	30.6
10	34.6	34.4	30.4	33.8
12	37.6	37.0	33.1	36.9
16	41.0	39.6	36.2	40.0
20	43.7	42.1	39.1	42.0
24	46.4	44.6	41.9	44.0
30	50.7	46.4	43.5	45.7
36	55.0	48.2	45.1	47.3
48	58.4	50.5	47.6	49.1
60	60.6	51.7	49.5	50.9
72	61.6	52.3	50.4	52.2
96	62.2	53.1	50.4	52.8

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 leguminous fodder crops

Fodder crop	Horsegram	Field bean	Cluster Bean	Cowpea
Kinetic parameters				
t _{1/2} (h)	11.66	8.203	8.588	7.57
k (h ⁻¹)	0.059	0.085	0.081	0.092
a	42.33	48.07	21.48	30.47
b	265.5	208.7	224.3	222.1
D ml	307.8	256.8	245.8	252.6
Gas at t _{1/2}	159.1	144.6	126.2	119.2
Substrate degradation (mg/g DM) at t_{1/2}				
TDOM	616.7	529.7	411.9	434.5
IVDMD	69.15	58.46	52.15	48.8
Microbial biomass synthesis indices at t_{1/2}				
PF	3.78	3.63	3.24	3.56
MBP	256.6	206.9	132.1	165.6
EMBS	414.5	389.9	320.2	381

Substrate degradation (TDOM) and Microbial biomass synthesis indices

The true digestible organic matter (TDOM) determined in the present study by RIVGPT was 616.7 (mg/g DM) in Horsegram which was significantly ($P < 0.01$) higher than other leguminous fodder crops. The lowest TDOM was observed in Cluster bean. 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, *et al.*, 2018) [10]. Partitioning factor is ratio of TDOM and gas produced at t_{1/2}. The PF was 3.78 in Horsegram, 3.63 in Field bean, 3.56 Cowpea and 3.24 in cluster bean which indicated the amount of organic matter

digested and diverted for microbial biomass production. The MBP (microbial biomass production) and efficiency of microbial biomass synthesis (EMBS) was significantly higher in Horsegram which had higher PF value. Similarly, as the PF value increased the MBP and EMBS were also increased in leguminous fodders where lowest MBP was noticed in Cluster bean.

IVDMD (%) of Cluster bean is similar to value reported by Datt *et al.* (2009) [7] and that of Cowpea was 19.9% lower than the value reported by Kumar *et al.* (2015) [14] which may be because of the fact that, degradability depends on rumen microorganisms having access to DM and OM (Ansah *et al.*, 2021) [1]. The comparatively high IVDMD of Horsegram indicate that it has better microbial access,

degradation, and fermentation than the other fodders. Every fodder examined in the present study exhibited a degradability level exceeding the minimum of 45% recommended for the purpose of sustaining cattle in tropical regions McDowell *et al.* (1972) [5].

PF for Cowpea observed in the present study is comparable with value as reported by Kumar *et al.* (2015) [14]. PF for Horsegram, Field bean, Cluster bean observed in the present study are within the range of values of 3.49 as reported by Biradar *et al.* (2007) [4] for Green gram straw to 4.55 as reported by Blümmel *et al.* (2003) [5] for Lucerne hay, which also belongs to same family (Fabaceae) as that of leguminous fodders examined.

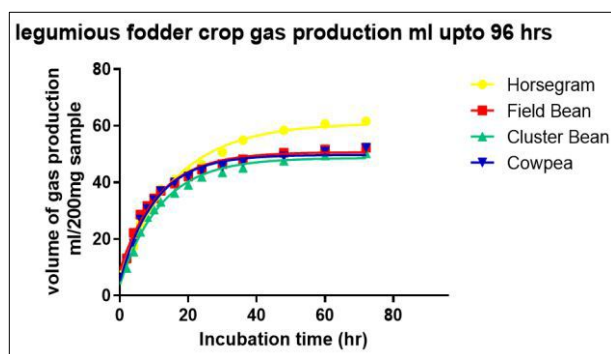


Fig 1: Rate of gas production (k) of leguminous annual fodder crops

Summary and Conclusion

The metabolizable energy (ME) values of the leguminous fodder crops, as determined by the rumen in vitro gas production technique (RIVGPT), ranged between 8 and 9 MJ/kg DM, with no significant variation observed among the different fodders. The rate of fermentation was approximately 9% per hour in cowpea, cluster bean, and field bean, indicating a relatively rapid degradability of these substrates in the rumen. An increase in partitioning factor (PF) values was associated with a corresponding increase in microbial biomass production, suggesting more efficient utilization of fermented substrates for microbial growth. Although the energy values and crude protein (CP) contents were comparable among the fodders, horsegram and field bean exhibited relatively higher microbial biomass production. Therefore, based on their superior efficiency in supporting microbial protein synthesis, horsegram and field bean can be recommended for inclusion in ruminant diets to enhance microbial protein yield, thereby contributing to improved total protein supply for growth and milk production.

Application of research

This *in vitro* study helps in understanding the level of nutrients in the leguminous annual fodder crops, the extent of nutrient digestibility and the predicted energy levels will help in formulating better diet for improved and economical production.

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