

Original Research Article

***In vitro* gas and methane production of Horse grass and Calopo at different forage to concentrate ratios in rabbits**

ABSTRACT

The *in vitro* gas production technique provides information about kinetics and fermentation characteristics of feedstuffs and diets, and it is a useful indicator of faecal microbial activity. **Aim:** The aim of this study was to use the *in vitro* technique to study gas production kinetics and dry matter degradability of feed samples at different forage to concentrate (F:C) ratios using rabbit inoculum. **Materials and Methods:** The treatments (T) were in a Completely Randomized Design with two forage sources namely Horse grass and Calopo mixed at 75:25 (T₁) ratio and F:C ratios 75:25 (T₂), 50:50 (T₃), 25:75 (T₄) and 100:0 (T₅), respectively. Six New Zealand White rabbits (mean body weight [BW] = 0.86kg ±0.04) were used as inoculum donors. **Results:** Crude protein of forages was 10.45 and 23.00 for Horse grass and Calopo respectively. The crude protein and crude fiber content of the supplementary concentrate was 17.01 and 6.00 respectively. Highest crude fiber (12.00) was also observed in Calopo. Diets 4 and 5 produced the highest (P<0.05) total gas and methane respectively, while *in vitro* degradability decreased with increasing supplementary concentrate (P<0.05). Sole forage diet resulted in highest dry matter degradability (6.90) and gas production (7.80). **Conclusion:** It can be concluded that good quality forage, therefore, could reduce the need for concentrate supplementation.

Keywords: Caecal fermentation, Degradability, Gas kinetics, Nutrient, Tropical forages.

1. INTRODUCTION

The rabbit (*Oryctolagus cuniculus*) caecum represents 40% of the total digestive tract by weight and accounts for about two-thirds of fecal fiber digestion (Merino and Carabaño, 1992). It is characterized by the presence of an abundant microbial community with wide microbial community with wide diversity and complex interactions (Rodrigo-Romero et al. 2011). Microbial community plays a role in feed digestion through fermentation and recycling of microbial protein through caecotrophy. The *in vitro* gas production technique has been used to evaluate the nutritive value of feed in caecal fermenters. It also provides information about kinetics and fermentation characteristics of feedstuffs and diets, and it is a useful indicator of faecal microbial activity (Stanco *et al.*, 2003). Therefore, the aim of this experiment was to investigate the effects of grass: legume mixed forages combined with different forage to concentrate ratios on dry matter degradation and gas production kinetics using the *in vitro* technique.

2. MATERIALS AND METHODS

2.1 Substrate preparation and analysis

Prior studies were carried out with rabbits to determine the acceptability of various forages. Horse grass and Calopo (75:25) were the most desirable and also improved performance. This informed the decision to use forages and concentrates as substrates as follows: sole forage (T₁: 75% Horse grass + 25% Calopo), Three concentrate to forage (T₂: 25% concentrate + 52.5% Horse grass + 22.5% Calopo; T₃: 50% concentrate + 35% Horse grass + 15% Calopo; and T₄: 75% concentrate + 17.5% Horse grass + 7.5% Calopo) and sole concentrate diet (T₅: 100% concentrate). Substrates were subsampled and initially weighed fresh on the field and then oven-dried to 65°C to constant weight. Dried samples were weighed and hammer-milled to pass through a 1mm sieve. Crude protein (% Nitrogen x 6.25), crude fiber, ash and ether extract were analyzed according to the standard methods of Association of Official Analytical Chemists (AOAC) (2000).

2.2 Animal donors and collection of caecal fluid

Six New Zealand White rabbits (mean body weight [BW] = 0.86kg ±0.04) were used as inoculum donors. The animals were previously fed commercial growers mash. Caecal fluid was collected in the morning in equal proportions from donor animals, under the same feeding regime post-slaughtering. Caecal contents collected from gastro-intestinal tract isolated after slaughtering were emptied into a pre-warmed thermos flask and strained through four-layered cheesecloth and kept at 39°C soon after collection. All laboratory handling of caecal fluid was carried out under a continuous flow of carbon dioxide (CO₂). The inoculum and a buffer solution were mixed in the ratio 1:2 (v/v), respectively as described by Menke and Steingrass (1988).

2.2 In vitro procedure

Oven-dried samples (0.2kg) were milled and accurately weighed into 100ml gas syringes fitted with plungers. *In vitro* incubation of the samples was conducted in triplicates. Syringes were filled with 30ml of medium consisting of 10ml of caecal fluid and 20ml of buffer solution. Three blanks containing 30ml of medium were incubated. The syringes were placed in rotor inside the incubator (39°C) with about one rotation per minute. The gas production was recorded after 3, 6, 9, 12, 18, and 48 h of incubation. The volume of methane gas produced by each diet was determined by dispensing 4ml of 10 N sodium hydroxide (NaOH) into each incubated sample at the end of the 48 h of incubation. NaOH was added to absorb carbon-dioxide produced during fermentation and the remaining volume of gas recorded as methane as described by Fievez *et al.* (2005).

2.3 Dry matter degradation, organic matter digestibility and metabolizable energy

The *in vitro* dry matter degradation was determined at 48 h by centrifuging the incubation residues at 20, 000 x g for 30 minutes following placement in iced cube (-4 °C) to end fermentation. Residues obtained were filtered and oven-dried to determine their dry weight. The blanks were also centrifuged and residues weighed and used to correct for residues from caecal inoculum. *In vitro* dry matter degradation was then calculated as:

$$\frac{\text{Substrate dry matter incubated} - \text{residue dry matter} - \text{blank dry matter}}{\text{Substrate dry matter incubated}}$$

Organic matter digestibility (OMD) and metabolizable energy (ME) was determined using the following equations as described by Menke and Steingrass (1988). $OMD = 14.88 + 0.88GV + 0.45CP + 0.651XA$. ME was determined using the equation: $ME = 2.20 + 0.136GV + 0.057CP + 0.00029CF$ (Menke and Steingrass, 1988). Where GV is total gas volume produced, CP is crude protein and CF crude fiber.

2.4 Data analysis

All data obtained were subjected to Statistical Analysis System (SAS, 2004) using a completely randomized design. For all parameters, differences between treatment means were contrasted by Duncan's new multiple range test (Steel and Terrie, 1980).

3. RESULTS

3.1 Chemical composition of experimental diets

At 45 day of growth, the dry matter, crude protein and crude fiber content (Table 1) of Horse grass and Calopo were 27.33, 10.45, 10.00% and 30.67, 12.00 and 10.00% respectively. The concentrate diet had a 17.01 and 6.00% CP and CF respectively. Horse grass and Calopo had the same (7.00%) ash content.

97 **Table 1: Feed ingredients and chemical compositions of concentrate and forages**

Feed ingredients	Percentage	Horse grass	Calopo
Maize offal	54.00		
Palm kernel cake	20.00		
Wheat offal	10.00		
Soybean meal	10.00		
Fish meal	2.50		
Bone meal	3.00		
Vitamin mineral premix	0.25		
Common salt	0.25		
Total	100.00		
Dry matter (%)	92.24	27.33	30.67
Crude protein (%)	17.01	10.45	12.00
Crude fiber (%)	6.00	10.00	10.00
Ether extract	5.30	4.10	5.70
Ash (%)	7.00	7.00	7.00
Nitrogen free extract (%)	64.69	68.45	75.20

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99 **3.2 Gas production kinetics and *in vitro* digestibility**

100 Gas production kinetics is shown in Table 2. They were significantly affected by diets. Gas production
101 level at 48 h was highest ($P<0.05$) in the diets with forage:concentrate (F:C) proportion of 75:25 (T_4)
102 and lowest in 25:75 F:C ratios (T_2). Methane production from incubated samples (Figure 1) showed
103 that diets 5 (6.10ml/200mgDM) and 3 (5.70ml/200mgDM) produced significantly the highest ($P<0.05$)
104 volume of methane. The soluble fraction 'a' of the samples was significantly higher ($P<0.05$) in diet
105 1(1.83) while other diets were the same (1.00). Potential gas production 'b' (ml/200mgDM) estimated
106 for T_1 (5.97) and T_2 (5.10) were statistically similar and lower than diets 3 (7.40) and 5 (7.20). The
107 fermentation rate constant 'c' of the insoluble fraction for all diets was not significantly ($P>0.05$)
108 different by treatments, although diet 1 was slightly higher than other diets. Sole forage (T_1) diets were
109 highest ($P<0.05$) in IVDMD (6.90) and OMD (31.87). The highest (4.48MJ/kg DM) and the least
110 (3.66MJ/kg DM) predicted ME was observed in T_1 and T_3 respectively.

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112 **Table 2. Gas production kinetics and *in vitro* degradability**

F:C ratios	Gas Production		Kinetics		Total Gas Production (ml/200mgDM) (48 h)	<i>In Vitro</i> Degradability IVDMD (%)	OMD (%)	ME (MJ/kg DM)
	A	B	a+b	C				
100:0	1.83 ^a	5.97 ^c	7.80 ^b	0.04	7.80 ^b	6.90 ^a	31.87 ^a	4.48 ^a
75:25	1.00 ^b	5.10 ^c	6.10 ^c	0.02	6.10 ^c	5.90 ^{ab}	27.30 ^{bc}	3.77 ^{bc}
50:50	1.00 ^b	7.40 ^b	8.40 ^b	0.02	8.40 ^b	5.45 ^{ab}	26.06 ^c	3.66 ^c
25:75	1.00 ^b	10.80 ^a	10.80 ^a	0.02	11.80 ^a	3.20 ^{bc}	28.39 ^{bc}	4.01 ^{bc}
0:100	1.00 ^b	7.20 ^b	8.20 ^b	0.02	8.20 ^b	2.20 ^c	28.77 ^{bc}	4.07 ^b
SEM	0.11 [*]	0.20 [*]	0.17 [*]	0.009 ^{ns}	0.17 [*]	0.56 [*]	0.41 [*]	0.06 [*]

113 abc: means along the same column with different superscripts are significantly different ($P<0.05$). * =
114 significant; ns = not significant; SEM: standard error of means; a = intercept (gas produced from
115 soluble fraction); b = gas production from insoluble fraction; c = gas production rate constant for
116 insoluble fraction (b); IVDMD = *in vitro* dry matter degradability; IVOMD = *in vitro* organic matter
117 digestibility.

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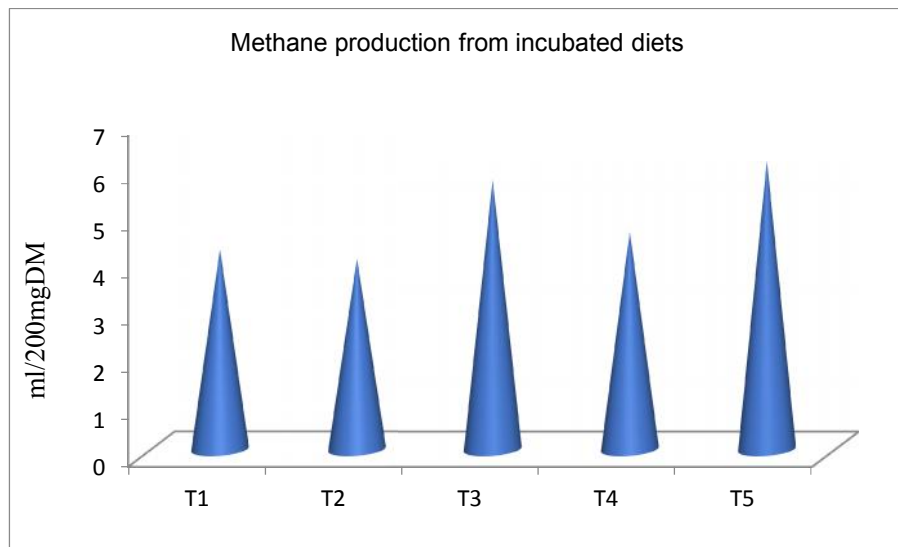


Figure 1: Methane production after 48 h incubation

4. DISCUSSION

Total gas production values obtained at 48 h in this study are lower than that reported by Khanum *et al.* (2007) but similar to the values reported by Njidda *et al.* (2010). These values depend to a large extent on the level and nature of fiber, presence of secondary metabolites and most importantly, the potency of the caecal/ruminal fluid used as inoculum (Babayemi *et al.*, 2006). It has been reported in most cases (Babayemi and Inyang, 2009; Babayemi and Bankole, 2009) that feedstuffs which show high capacity for gas production are also observed to be synonymous with high methane production. The results of this study confirmed this assertion. Dry matter degradability values obtained in his study is similar to values reported by Njidda *et al.* (2010) but at variance with values of Khanum *et al.* (2007). This could be as a result of the level and nature of fiber, presence of secondary metabolites and most importantly, the potency of the caecal/ruminal fluid used as inoculum as reported by Babayemi *et al.* 2006. The predicted difference of ME and OMD of diets reflected contents of fermentable carbohydrate and available nitrogen (Menke and Steingass, 1988).

5. CONCLUSION

Sole forage diet resulted in highest dry matter degradability and gas production. It may be suggested that good quality forage could reduce the need for concentrate supplementation. Further research using Horse grass and Calopo to improve caecal fermentation and feed efficiency in rabbits are recommended.

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