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Use of an in vitro gas production method to investigate interactions between veld hay and Napier hay or groundnut hay supplements

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Abstract

Measurement of gas produced during in vitro fermentation was used to investigate the fermentability of poor quality natural pasture (veld) hay mixed with different amounts of Napier hay or groundnut hay. In vitro fermentations were conducted in nitrogen-rich (NR) and nitrogen-free (NF) media. Groundnut hay was more rapidly fermented than Napier hay, the nitrogen content of the medium making little difference to the fermentation characteristics of either hay. Veld hay was the least fermentable substrate, particularly when NF medium was used. Statistically significant positive interactive effects were observed between both supplements and veld hay fermented in both media as gauged by gas production and dry matter disappearance. Evidence of significant interactions had not been obtained from earlier measurements of in vivo digestibility using the same feeds, but they may have been obscured by increased rates of passage with increased supplementation. Differences between gas productions in NR and NF media were explored as possible indicators of nitrogen deficiency in feed mixtures. Both Napier hay and groundnut hay appeared to be balanced in terms of energy and nitrogen fermentable by rumen microbes. © 1997 Elsevier Science B.V.

Keywords: In vitro gas production; Feed interactions; Supplementation

1. Introduction

Feeds are usually assessed for nutritive value singly, not as the mixtures of feeds which animals generally consume. The nutritive values of the feeds in mixtures are

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assumed to be additive. For ruminant feeds such evaluation can be misleading as one forage can influence another in terms of digestion and nitrogen utilisation (Moss et al., 1992). In less developed countries, where dry season feeds are often deficient in nitrogen and high quality feeds are in short supply, interactions between feeds may be particularly important.

One area of interest is the use of supplements to forages which are high in fibre and low in nitrogen. Silva and Ørskov (1988) concluded that supplementation with feeds providing digestible cellulose and/or hemicellulose improved the conditions in the rumen for barley straw fibre degradation, increasing both the rate and extent of degradation of straw. The protein supplement soya bean meal did not appear to affect straw degradation in the rumen, while fish meal led to some improvement in straw degradation, but in these trials diets were also supplemented with urea to ensure that they were not nitrogen-deficient. Silva et al. (1989) reported that supplementation of barley straw with fish meal and/or sugar-beet pulp increased in vivo apparent digestibility and live-weight gains, and tended to increase the intake of barley straw by sheep and cattle. Diets were also supplemented with urea to ensure that they were not nitrogen-deficient for these studies. Manyuchi (1994) and Manyuchi et al. (1996) investigated the supplementation of poor quality natural pasture (veld) hay with Napier hay and groundnut hay, supplements potentially available to many livestock keepers in Zimbabwe, concluding that these supplements increased the intake of veld hay. However, feed interactions remain poorly understood and difficult to investigate.

The production of gas has been used as a measure of the in vitro degradation of feeds by rumen micro-organisms (Theodorou et al., 1994). This approach has been used to investigate interactions between feeds, where interactions have been defined as the differences between the gas production from substrate mixtures and that predicted from the gas productions of the substrates when fermented alone (Prasad et al., 1994). Statistically significant interactive effects have been observed between finger millet straw and various supplements (Prasad et al., 1994; Sampath et al., 1995), with similar trends observed in both in vitro gas production and in vivo digestibility (Prasad et al., 1994). The in vitro gas production method of Theodorou et al. (1994), therefore, appeared to be suitable for investigating the rates and extends of fermentation of feed mixtures.

The Theodorou technique usually uses a nitrogen-rich (NR) medium, the nitrogen being mainly in the form of ammonium bicarbonate, so that fermentation rates are not determined by the nitrogen availability of the feed. This is to indicate the value of the feed when fed as part of a balanced diet, but may not relate to the nitrogen-deficient diets often fed to livestock in less developed countries. The nitrogen in the medium can act as a supplement to nitrogen-deficient feeds (or feed mixtures), increasing the rate of gas production in the in vitro system used for this study (Dryhurst and Wood, 1997). Therefore a nitrogen-free (NF) medium, or the use of both NF and NR media, may be more suitable for investigating the supplementation of forages which are nitrogen deficient. Both NF and NR media were used to evaluate their relative merits in this type of study. The samples were taken from feeds used by Manyuchi (1994) and Manyuchi et al. (1996) in trials in vivo, and the levels of supplementation used in vitro were selected to be similar to the in vivo trial.

2. Materials and methods

Samples of veld hay, Napier hay and groundnut hay were taken from the feeds used for the in vivo feeding trials described by Manyuchi (1994) and Manyuchi et al. (1996). Their composition is given in Table 1. Veld hay had been harvested in the dry season, Napier hay harvested at 4 weeks growth during the wet season and groundnut hay harvested when the crop was mature.

The in vitro gas production method of Theodorou et al. (1994) was used. This involved the anaerobic fermentation of dried substrate using inoculum prepared from fresh rumen fluid. NR medium containing 160 mg l^{-1} nitrogen (140 mg l^{-1} as ammonium bicarbonate and 20 mg l^{-1} from cysteine in the reducing agent) was used in experiment 1. NF medium described by Menke et al. (1979) was used in experiment 2. Additionally, in experiment 1, groundnut hay and Napier hay were fermented in the NF medium, as well as NR medium.

For all treatments a total of 1 g of substrate (veld hay + supplement) was used. The levels of supplementation used in vitro were selected to be similar to the in vivo trial (specifically experiment 1 of Manyuchi, 1994; Manyuchi et al., 1996, which excluded urea from the diets). The same range of inclusion of supplements was used for both in vitro experiments 1 and 2. In experiment 1 all treatments were fermented in triplicate for 166 h and selected treatments (the single substrates and the mixtures most closely corresponding to in vivo data) for 52 h (also in triplicate). Prasad et al. (1994) indicated that in vitro and in vivo digestibilities were most similar after about 52 h of incubation. The inclusion rates of the supplements used are given in Tables 2 and 3.

In experiment 2 all treatments were incubated for 70 h in triplicate, with single substrates being further replicated to give six analytical replicates. Experiment 1 had indicated that the best fits between in vitro and in vivo data were generally between 44 and 66 h of incubation (estimated as described below). An incubation period of 70 h was selected, slightly longer than this range so as to obtain the most relevant gas production and DMD data. At the end of the fermentation period, samples were filtered into preweighed filter crucibles (porosity P160; British Standard grade 1), dried and weighed, and dry matter disappearance (DMD) calculated.

Table 1
Chemical composition, in vitro and in vivo digestibility of veld hay, Napier hay and groundnut hay (taken from Manyuchi, 1994)

Component (g kg^{-1} DM)	Veld hay	Napier hay	Groundnut hay
Ash	115	65	70
Nitrogen	3.8	22.1	21.0
Neutral detergent fibre (NDF)	848	700	455
Acid detergent fibre (ADF)	524	329	330
Nitrogen in ADF (g kg^{-1})	2.4	4.6	7.7
In vitro DM digestibility [a] (g kg^{-1})	0.38	0.67	0.65
In vivo DM digestibility (g kg^{-1})	0.40	0.66	0.56

[a] In vitro digestibility by method of Tilley and Terry (1963).

Table 2
Mean values of cumulative gas production (CG) and dry matter disappearance (DMD) at different incubation periods: Veld hay supplemented with Napier hay in nitrogen rich (NR) medium

Weight substrate (g)		Gas production parameter				
Veld hay	Napier hay	CG12 [ab]	CG52	CG166	DMD52 [c]	DMD166
1.00	0.00	30.0 (6) [d]	135.8 (6)	250.1 (3)	0.432 (3)	0.604 (3)
0.90	0.10	29.2 (3)	151.8 (3)	246.0 (3)	nd	0.645 (3)
0.79	0.21	34.6 (6)	160.2 (6)	254.9 (3)	0.507 (3)	0.684 (3)
0.76	0.24	31.2 (3)	164.1 (3)	245.3 (3)	nd	0.663 (3)
0.70	0.30	36.7 (3)	176.1 (3)	253.9 (3)	nd	0.663 (3)
0.61	0.39	36.6 (6)	168.8 (6)	251.0 (3)	0.551 (3)	0.680 (3)
0.50	0.50	42.9 (3)	187.9 (3)	256.7 (3)	nd	0.720 (3)
0.38	0.62	42.1 (6)	185.5 (6)	259.4 (3)	0.623 (3)	0.727 (3)
0.20	0.80	40.8 (3)	190.0 (3)	255.4 (3)	nd	0.762 (3)
0.00	1.00	44.3 (6)	184.6 (6)	247.0 (3)	0.724 (3)	0.781 (3)
	SE (3) [e]	3.26	6.03	4.38	0.0027	0.0115
	SE (6)	2.31	4.26			

[a] CG = cumulative gas production g^{-1} dry matter.
[b] Numbers indicated the length of incubation (h).
[c] DMD = dry matter disappearance measured gravimetrically.
[d] Numbers in parenthesis denote number of replicates.
[e] SE (3), (6) standard error of means for 3 or 6 replicates
nd: not determined.

2.1. Computation of data and statistical analysis

Data were analysed as described by Prasad et al. (1994) to obtain estimates of incubation times which appeared to most closely relate to the in vivo situation. These times were used to help select incubation times for the analysis of interactive effects

Table 3
Mean values of cumulative gas production and dry matter disappearance (DMD) at different incubation periods: Veld hay supplemented with groundnut hay in nitrogen rich (NR) medium

Weight substrate (g)		Gas production parameter				
Veld hay	Groundnut hay	CG12 [ab]	CG52	CG166	DMD52 [c]	DMD166
1.00	0.00	30.0 (6) [d]	135.8 (6)	250.1 (3)	0.432 (3)	0.604 (3)
0.90	0.10	42.7 (3)	163.4 (3)	260.5 (3)	nd	0.640 (3)
0.81	0.19	49.7 (6)	173.0 (6)	260.6 (3)	0.550 (3)	0.655 (3)
0.68	0.32	47.8 (6)	170.3 (6)	255.2 (3)	0.586 (3)	0.658 (3)
0.58	0.42	67.1 (3)	193.6 (3)	247.7 (3)	nd	0.675 (3)
0.54	0.46	68.1 (6)	189.2 (6)	251.9 (3)	0.635 (3)	0.679 (3)
0.40	0.60	69.3 (3)	195.8 (3)	245.2 (3)	nd	0.655 (3)
0.35	0.65	85.1 (6)	201.3 (6)	251.8 (3)	0.666 (3)	0.679 (3)
0.15	0.85	92.2 (3)	215.4 (3)	252.7 (3)	nd	0.720 (3)
0.00	1.00	108.0 (6)	213.7 (6)	240.4 (3)	0.719 (3)	0.720 (3)
	SE (3) [e]	5.03	6.66	4.30	0.0028	0.0059
	SE (6)	3.56	4.71			

See Table 2 for notes.

described below. The following single pool exponential equation of Merry et al. (1991), described by Prasad et al. (1994), was fitted to the cumulative gas production data:

$$\text{Volume} = a + b(1 - \exp(-kt))$$

where constants a, b and k are a scale factor, the gas pool size and the rate constant, respectively. Data from 12 h fermentation onwards only were used.

Using data from the gas production from 1 g of each individual feed, a predicted value for the gas production (assuming no interactions) from the substrate mixtures was obtained. This was calculated by summing the gas produced from the relevant quantity of individual substrates (veld hay and Napier or groundnut hay) assuming a linear relationship between quantity of substrate and gas production. This relationship had been shown to be linear over the range 0.0 to 2.0 g for perennial ryegrass (Theodorou et al., 1994), horse-chestnut leaves and barley straw (C.D. Wood, unpublished data, 1992). An interaction was defined as the difference between a predicted parameter and that measured experimentally from substrate mixtures. The pooled standard errors of the means of the gas production and DMD for substrate mixtures were calculated. The statistical significance of interactive effects was evaluated by comparing this predicted value with the experimental mean values and their pooled standard errors. For ease of interpretation differences (interactions) were calculated as a percentage of the predicted value.

The differences in cumulative gas production (diff CG) in NR and NF media with different levels of supplementation were calculated.

3. Results

3.1. Gas production characteristics

The cumulative gas (CG) production curves for veld, Napier and groundnut hays when fermented alone are presented in Fig. 1. The gas produced after 52 h incubation of Napier and groundnut hays was 184.6 and 213.7 ml g^{-1} DM (standard errors 4.26 and 4.71), respectively, in experiment 1, NR medium. For these substrates no significant differences ($P > 0.05$) were found between gas production in NR and NF media in experiment 1. In experiment 2, 52 h gas productions of 207.8 and 245.1 ml g^{-1} DM (standard errors 4.83 and 6.40) were found for Napier and groundnut hays respectively in NF medium, significantly ($P < 0.05$) higher than the same substrate/medium combinations in experiment 1. The differences between experiments were apparently due to differences in the inocula used.

Veld hay was fermented very much more quickly in the NR medium compared with NF medium, 135.8 and 57.0 ml g^{-1} DM respectively after 52 h incubation, differences significant $P < 0.001$. The low gas production from veld hay in NF medium was apparently due to the medium and not to differences in inocula, and indeed the differences between the inocula used for experiments 1 and 2 would have tended to reduce the observed differences between the media.

Estimated times of best fit with in vivo data averaged 62 h for veld hay/Napier hay mixtures in NR medium, 96 h in NF medium. For veld hay/groundnut hay mixtures the corresponding times of best fit were 53 and 54 h. For veld hay in NF medium and

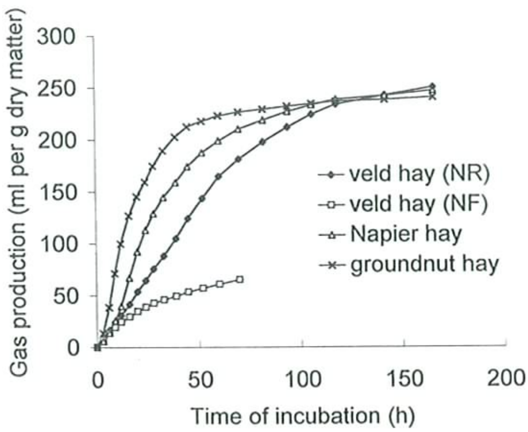

Fig. 1. Cumulative gas production with time for the three feeds fermented separately in NR medium (nitrogen-rich) and NF medium (nitrogen-free). Curves for Napier and groundnut hays were very similar in both media, data using NR medium only is shown here.

Napier hay in NR medium no values were obtained as the in vitro degradation projected by the fitted exponential curve did not reach the value of the in vivo digestibility.

3.2. Fermentability of feed mixtures

The mean gas production and DMD at selected times of incubation are given in Tables 2–5 (for Napier and groundnut hay in NR and NF media) together with the pooled standard errors of the means. These data were used to derive the values for the

Table 4
Mean values of cumulative gas production and dry matter disappearance (DMD) at different incubation periods: Veld hay supplemented with Napier hay in Nitrogen free (NF) medium

Weight substrate (g)		Gas production parameter			
Veld hay	Napier hay	CG12 [ab]	CG52	CG70	DMD70 [c]
1.00	0.00	24.4 (6) [d]	57.0 (6)	65.2 (6)	0.165 (6)
0.90	0.10	25.6 (3)	72.9 (3)	86.0 (3)	0.233 (3)
0.79	0.21	27.1 (3)	89.3 (3)	109.3 (3)	0.307 (3)
0.76	0.24	26.8 (3)	92.7 (3)	115.5 (3)	0.334 (3)
0.70	0.30	26.8 (3)	101.0 (3)	126.2 (3)	0.381 (3)
0.61	0.39	31.5 (3)	130.8 (3)	164.3 (3)	0.464 (3)
0.50	0.50	32.8 (3)	140.0 (3)	173.0 (3)	0.511 (3)
0.38	0.62	30.4 (3)	151.4 (3)	187.1 (3)	0.541 (3)
0.20	0.80	40.5 (3)	194.1 (3)	223.8 (3)	0.650 (3)
0.00	1.00	42.5 (6)	207.8 (6)	233.0 (6)	0.699 (6)
	SE (3) [e]	3.37	6.83	7.12	0.0152
	SE (6)	2.38	4.83	5.03	0.0107

See Table 2 for notes.

Table 5
Mean values of cumulative gas production and dry matter disappearance (DMD) at different incubation periods: Veld hay supplemented with groundnut hay in nitrogen free (NF) medium

Weight substrate (g)		Gas production parameters			
Veld hay	Groundnut hay	CG12 [ab]	CG52	CG70	DMD70 [c]
1.00	0.00	24.4 (6)	57.0 (6) [d]	65.2 (6)	0.165 (6)
0.90	0.10	37.6 (3)	87.8 (3)	103.1 (3)	0.250 (3)
0.81	0.19	42.8 (3)	107.5 (3)	127.8 (3)	0.324 (3)
0.68	0.32	48.7 (3)	129.6 (3)	153.1 (3)	0.387 (3)
0.58	0.42	52.7 (3)	145.8 (3)	171.9 (3)	0.442 (3)
0.54	0.46	64.6 (3)	178.1 (3)	208.3 (3)	0.533 (3)
0.40	0.60	68.5 (3)	176.4 (3)	202.0 (3)	0.564 (3)
0.35	0.65	75.0 (3)	189.9 (3)	214.1 (3)	0.588 (3)
0.15	0.85	95.5 (3)	218.1 (3)	237.6 (3)	0.658 (3)
0.00	1.00	108.3 (6)	245.1 (6)	260.0 (6)	0.702 (6)
	SE (3) [e]	6.51	9.05	9.49	0.0129
	SE (6)	4.60	6.40	6.71	0.0091

See Table 2 for notes.

percentage differences between predicted and observed gas production and DMD given in Tables 6 and 7 for NR medium and NF medium, respectively.

For the incubations conducted using NR medium, interactive effects were generally at their highest between 33 and 52 h incubation (selected data presented in Fig. 2). No general trend nor statistically significant ($P > 0.05$) interactions in gas production were observed at 12 h incubation for mixtures containing either Napier or groundnut hay. At 52 h positive interactive effects on gas production were observed for all levels of inclusion of Napier or groundnut hays. With Napier hay, these differences were statistically significant ($P < 0.05$) for inclusion rates of 0.21 to 0.80 g g^{-1}. These increases in gas production reached 17.1% and 17.3% at 0.30 and 0.50 g g^{-1} Napier hay, respectively. With groundnut hay mixtures, interactive effects on gas production at 52 h incubation reached 14.9% at inclusion rates of 0.19 and 0.42 g g^{-1}, although interactions did not achieve statistical significance over the full range of mixtures. Thus, the extends of the interactive effects were similar with both Napier and groundnut hays, with some indication that maximum interaction was achieved with a lower level of groundnut hay than Napier hay. At 166 h incubation, interactions were generally positive but much lower (and generally not statistically significant, $P > 0.05$) than at 52 h, reaching maxima of 4.5% and 5.0% with Napier hay and groundnut hay, respectively.

For DMD of Napier hay mixtures, 52 h interactive effects were much lower than those observed in gas production. For example, with inclusion of 0.62 g g^{-1} of Napier hay, gas production was increased by a highly significant ($P < 0.001$) 11.7% above that predicted without interactions, while DMD was only 1.6% higher ($P > 0.05$). In contrast, for groundnut hay mixtures, positive interactions were very similar whether they were measured by gas production or DMD. The only major difference was that, at 0.32 g g^{-1} of groundnut hay, the 11.9% increase in DMD appeared to be a more reliable indicator than the 6.0% increase in gas production, which looked anomalously low compared with the trend of the gas production data. At 166 h, interactive effects in

Table 6
Differences (%) [a] between gas production and dry matter disappearance observed for supplemented veld hay and that predicted from veld hay and supplement fermented separately in nitrogen rich (NR) medium (Experiment 1)

Veld hay + Napier hay	Parameter measured				
Level of inclusion of supplement (g per g^{-1} substrate)	CG12 [bc]	CG52	CG166	DMD52 [d]	DMD166
0.10	−7.1	7.9	−1.5	nd	3.7
0.21	4.8	9.7 *	2.2	2.8 *	6.7 **
0.24	−6.7	11.2 *	−1.6	nd	2.6
0.30	7.0	17.1 ***	1.9	nd	0.9
0.39	2.9	9.0 *	0.8	0.9	1.0
0.50	15.5	17.3 ***	3.3	nd	4.0
0.62	8.3	11.7 ***	4.5 *	1.6	1.9
0.80	−1.5	8.7 *	3.1	nd	2.2
Veld hay + groundnut hay					
0.10	13.0	13.8 *	4.6	nd	4.0 **
0.19	10.9	14.9 ***	5.0 *	13.0 ***	4.6 **
0.32	−13.0	6.0	3.3	11.9 ***	2.6 *
0.42	6.9	14.9 **	0.7	nd	3.4 **
0.46	3.4	10.2 **	2.5	12.6 ***	3.3 **
0.60	−9.8	7.3	0.4	nd	−2.8 *
0.65	5.5	8.0 *	3.3	7.7 **	−0.1
0.85	−4.3	6.6	4.5	nd	2.5 *

[a] Difference(%) = (observed gas production − predicted gas production)/(predicted gas production) × 100
[b] CG = cumulative gas production g^{-1} dry matter.
[c] Numbers indicated the length of incubation in hours.
[d] DMD = dry matter disappearance measured gravimetrically. Difference (%) calculated as in note a using DMD instead of gas production.
nd: not determined.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DMD were generally positive and low, although for groundnut hay most were statistically significant.

When the NF medium was used, there were increased interactive effects as the period of incubation increased. Generally negative (but not significant, $P > 0.05$) interactive effects on gas production were observed at 12 h with Napier hay mixtures. At 52 h, the trend was mainly positive, but not usually significant ($P > 0.05$). At 70 h, interactive effects were positive and statistically significant ($P < 0.05$) for inclusion of 0.39 g g^{-1} or higher of Napier hay; the gas production was increased by 10.6% to 25.8% compared to the no-interaction predicted value. Interactive effects observed in gas production at 70 h incubation were broadly mirrored by those observed in DMD.

For groundnut hay mixtures using NF medium, the overall picture was similar to that observed for Napier hay. Interactive effects became increasingly positive as incubation proceeded up to 70 h. Statistically significant ($P < 0.05$) interactions were observed from groundnut hay inclusion rates of 0.19 g g^{-1} up to a maximum increase of 34.6% in gas production at 0.46 g g^{-1} substrate. As with Napier hay mixtures, the DMD data broadly mirrored the interactions observed by gas production.

Table 7
Differences (%) [a] between gas production and dry matter disappearance observed for supplemented veld hay and that predicted from veld hay and supplement fermented separately in nitrogen free (NF) medium (Experiment 2)

Veld hay + Napier hay	Parameter measured			
Level of inclusion of supplement (g per g^{-1} substrate)	CG12 [bc]	CG52	CG70	DMD70 [d]
0.10	−2.3	1.1	4.9	6.7
0.21	−3.9	0.7	8.8	10.8
0.24	−6.8	−0.5	9.5	13.9 *
0.30	−10.2	−1.2	9.2	17.2 **
0.39	0.1	12.9	25.8 ***	24.3 ***
0.50	−1.9	5.7	16.0 **	18.3 ***
0.62	−14.7	0.6	10.6 *	9.1 *
0.80	4.2	9.3 *	12.2 **	9.8 **
Veld hay + groundnut hay				
0.10	14.7	15.8	21.8	14.3
0.19	6.1	15.9	25.0 *	21.3 ***
0.32	−5.0	10.6	20.0 *	14.9 **
0.42	−11.6	7.2	16.9 *	13.2 **
0.46	2.5	24.1 **	34.6 ***	29.4 ***
0.60	−8.3	3.9	10.9	15.8 ***
0.65	−5.0	5.9	11.6 *	14.4 ***
0.85	−0.2	0.6	3.0	5.9 *

For notes see Table 6.

3.3. Differences in gas production in NR and NF media (medium nitrogen effect)

Patterns of differences in gas production in NR and NF media, the medium nitrogen effect, were very similar with both supplements as illustrated by the selected data given

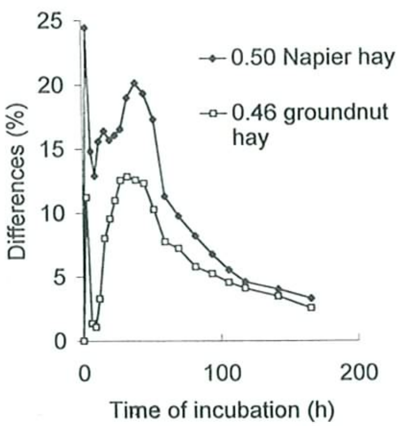

Fig. 2. Differences (interactions, %) at different times of incubation for 0.50 g Napier hay + 0.50 g veld hay and 0.46 g groundnut hay + 0.54 g veld hay. (Difference (%) = 100 × (observed gas production − predicted gas production)/(predicted gas production)).

Table 8
Difference (%) in gas production from veld hay supplemented with Napier or groundnut hays fermented in nitrogen rich (NR) and nitrogen free (NF) media at selected incubation times (Experiments 1 and 2, respectively)

Napier hay supplement	Differences (%)			
Wt supplement ($g\ g^{-1}$ substrate)	CG12 [ab]	CG24	CG52	CG70
0	6.5	26.0	86.4	115.7
0.1	3.7	23.8	79.0	100.9
0.21	8.4	31.9	80.5	92.3
0.24	4.4	26.5	71.5	79.3
0.3	9.8	35.1	75.1	79.7
0.39	6.6	24.3	48.0	42.1
0.5	10.1	30.4	47.9	41.5
0.62	11.4	33.2	40.8	31.8
0.8	0.3	15.0	−4.0	−8.6
1.0	−2.7	4.8	−20.7	−23.0
Groundnut hay supplement				
Wt supplement ($g\ g^{-1}$ substrate)	CG12	CG24	CG52	CG70
0	6.5	26.0	86.4	115.7
0.1	5.1	21.6	75.7	95.1
0.19	7.4	28.0	70.6	80.2
0.32	6.6	27.0	55.8	57.1
0.42	14.4	34.0	47.8	39.4
0.46	2.9	17.0	16.4	8.0
0.6	0.9	18.9	19.4	13.3
0.65	5.8	21.0	16.2	9.6
0.85	−3.3	10.6	−2.7	−9.9
1.0	−8.4	−5.5	−27.5	−33.7

[a] CG = cumulative gas production g^{-1} dry matter.
[b] Numbers indicated the length of incubation (h).

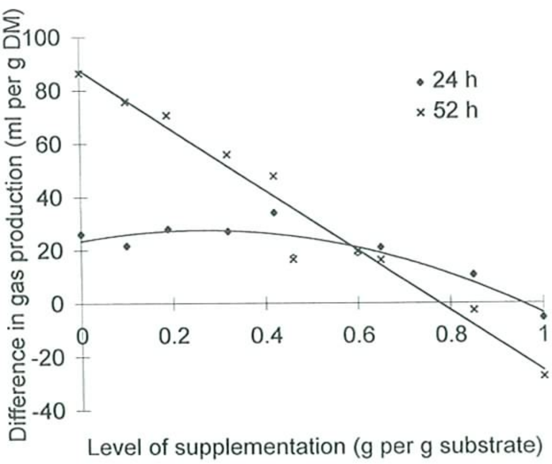

Fig. 3. Difference in cumulative gas production after 24 and 52 h incubation in nitrogen rich (NR) and nitrogen free (NF) media (medium nitrogen effect) for veld hay supplemented with groundnut hay. (Medium nitrogen effect = cumulative gas produced in NR medium − cumulative gas produced in NF medium).

in Table 8. Differences were low after 12 h incubation at all supplementation levels, but increased progressively from this incubation time. Fig. 3 illustrates the relationship between the medium nitrogen effect at 24 and 52 h incubation for veld hay supplemented with groundnut hay. For Napier hay the relationships were closely similar. At 24 h incubation, the medium nitrogen effect did not appear to be affected by supplementation up to levels of 0.62 and 0.65 g g^{-1} substrate for Napier and groundnut hays respectively. At 52 h of incubation (and beyond) there was a linear relationship between level of supplementation and medium nitrogen effect, the best fitting linear equations being:

Napier hay (at 52 h of incubation)

$$\text{medium nitrogen effect} = -112.9\,\text{supplement}\,(\text{g g}^{-1}\,\text{substrate}) + 97.4\ (R^2 = 0.938)$$

groundnut hay (at 52 h of incubation)

$$\text{medium nitrogen effect} = -112.9\,\text{supplement}\,(\text{g g}^{-1}\,\text{substrate}) + 87.6\ (R^2 = 0.959)$$

4. Discussion

4.1. Gas production characteristics

Groundnut hay was fermented relatively quickly, followed by Napier hay and veld hay. These fermentation characteristics were consistent with digestibility measurements reported by Manyuchi (1994). In vitro gas production technique was sensitive to the nitrogen levels of the medium when the nitrogen-poor veld hay was fermented. Napier and groundnut hays appeared to provide sufficient nitrogen to the micro-organisms to prevent it from being limiting, hence the lack of differences when these substrates were fermented in NR and NF media. Satter and Slyter (1974) indicated that 20–50 mg l^{-1} ammonium nitrogen was required to ensure maximum microbial growth in vitro. Recent trials have demonstrated that the in vitro gas production system used here was inhibited if nitrogen concentrations were below 50 to 100 mg l^{-1} (Dryhurst and Wood, 1997). Veld hay could only provide 1.2 mg nitrogen g^{-1} substrate fermented in 100 ml medium, giving 12 mg l^{-1} nitrogen in the medium, assuming nitrogen in the NDF was not available to the micro-organisms.

4.2. Fermentability of feed mixtures

Both supplements were able to stimulate the activity of rumen micro-organisms to produce in vitro interactive effects when fermented with veld hay. In NR medium, interactive effects were at their highest between 33 and 52 h. Both this study and that of Prasad et al. (1994) indicated that in vitro incubation for 52 h promoted digestion similar to that in the rumen. Conversely, the decrease in interactive effects at later incubation times may not be relevant to the physiological situation.

In NF medium, interactive effects occurred at later incubation times than in the NR medium. In general, veld hay was fermented much more slowly in NF medium than in the NR medium. Fermentation in the NR medium appeared to correspond more closely to the situation in vivo than that observed in the NF medium, presumably because ruminants are able to supply nitrogen to the rumen. It was, however, of note that interactions were observed in both media, so they were not completely suppressed by inorganic nitrogen. The interactions were, therefore, presumably due to the supply by the supplements of readily fermentable material as energy substrates and/or the supply of amino-acids and peptides to support rapid microbial growth. The interactions were probably similar in nature to those observed by Silva and Ørskov (1988) between unmolassed sugar-beet pulp or dried grass and untreated barley straw, where interactions were thought to be due to the stimulation of the growth of cellulolytic bacteria by the provision of readily digestible cellulose and/or hemicellulose by the supplement. However, there was no direct evidence from this study to distinguish the relative importance of energy and amino acid supply in causing the interactions.

Gas production gives a measure of the degradation of substrate, particularly the carbohydrate fraction (Menke et al., 1979). Methods which measure the loss of material not retained by some type of physical filter, such as the DMD method used here, give a measure of the solubility and solubilisation of the substrate. Lack of correlation between gas production and DMD could be due to soluble substrate not being fully degraded to gas and/or to variability in the stoichiometry of gas production. There was broad consistency between the extent of the interactions measured by gas production and DMD. This indicated that the interactions were largely, if not entirely, due to an increase in the rate of degradation of insoluble substrate. The reasons for the low interactions in DMD52 for Napier hay supplementation in NR medium compared to CG52 are unclear.

Manyuchi (1994) found a shift towards lower acetate and higher propionate production with supplementation in in vivo trials (molar proportions, veld hay: acetate 76.6%, propionate 17.1%; napier hay: acetate 65.7%, propionate 24.0%; groundnut hay: acetate 65.3%, propionate 24.4%). From theoretical considerations, the stoichiometry of acetate and propionate type of fermentations are:

Acetate: 1 mol carbohydrate yields 1.53 mol gas ($CH_4 + CO_2$) + 1.8 mol volatile fatty acids (VFAs).

Propionate: 1 mol carbohydrate yields 1.30 mol gas + 1.8 mol VFAs.

Assuming 1 mol VFA releases 1 mol CO_2 from the buffer, for veld hay 1 mol carbohydrate would yield 3.08 mol gas while for Napier hay 2.92 mol gas would be released. Shifts in stoichiometry of gas production due to changes in the molar proportions of VFAs produced could account for differences in stoichiometry of about 5% for these feeds. Thus non-linear shifts in stoichiometry resulting in higher than expected acetate production from veld hay/Napier hay mixtures, if they occurred, could account to some extent for the interactions in gas production not being reflected in interactions in DMD52. However, the molar VFA proportions for both supplements were similar, perhaps indicating that it was some other property of Napier hay/NR medium combination that led to the low interaction in DMD52 in NR medium. Possibly increased microbial production in the NR medium led to an increased contamination of the residue, leading DMD to underestimate the extent of degradation.

No interactive effects on digestibility were observed in vivo with either supplement. However, both Napier and groundnut hay supplementation stimulated the intake of veld hay (Manyuchi, 1994). Faecal dry matter excretion also increased as feed intake increased, while the digesta pool size was not altered, indicating a greater passage rate of digesta (Manyuchi, 1994; Manyuchi et al., 1996). This is consistent with a faster rate of rumen degradation leading to reduced rumen retention times, an increased rate of passage, but no apparent interactive effects on in vivo digestibility (Manyuchi, 1994). In sacco degradation of veld hay was largely unaltered by supplementation (Manyuchi, 1994), although for the in sacco experiments the diets were supplemented with 1% urea which may have suppressed a major effect of the Napier and groundnut hay supplements on the rumen environment. Manyuchi et al. (1996) suggested that the 1% urea supplement used probably provided adequate nitrogen to the rumen microbes to prevent it from being rate limiting. A possible alternative explanation is that increased hindgut fermentation in response to supplementation could compensate for the effect of increased passage rates on rumen degradation (Manyuchi, 1994). This in vitro study suggests that interactive effects on the rate of degradation may occur in the rumen.

4.3. Differences in cumulative gas production with and without N in the medium (medium nitrogen effect)

The lack of effect of supplementation on medium nitrogen effect until the inclusion of relatively high levels of supplement (over 0.6 g g^{-1} substrate) after 24 h incubation is notable. This indicated that low levels of supplement were not alleviating the nitrogen deficiency of the substrate at that incubation time. The data are consistent with the view that the supplements and veld hay have some carbohydrate components which can be fermented relatively quickly if nitrogen is available. It is suggested that in NF medium, degradation of supplement protein limited the rate of degradation of the rapidly fermentable carbohydrate.

The later linear relationships between medium nitrogen effect and supplementation level indicate that neither Napier hay nor groundnut hay was providing excess nitrogen, but both supplements were balanced in terms of protein and carbohydrate fermentable by rumen microbes. Dryhurst and Wood (1997) found that there was little response to added nitrogen in the medium after 52 h incubation at levels over 80 mg l^{-1} in the medium. This would indicate that for both supplements about half of the crude protein content was available to the rumen microbes in vitro. The negative values at high supplement levels relate to differences in the inocula between experiments 1 and 2.

5. Conclusions

Positive interactions were found during the in vitro fermentation of veld hay supplemented with either Napier hay or groundnut hay. Similar interactions had not been observed during in vivo digestibility measurements made using the same feed mixtures. This may have been due to rates of passage increasing with supplementation compensating for an increased rate of digestion, giving an apparent lack of interaction in in vivo digestibility. Both Napier hay and groundnut hay appeared to be sufficient in nitrogen fermentable by rumen microbes.

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References

Dryhurst, N., Wood, C.D., 1997. The effect of nitrogen source and concentration on in vitro gas production using rumen micro-organisms. Anim. Feed Sci. Technol. submitted.

Manyuchi, B., 1994. High quality forages as supplements to low quality forages for ruminants. Effects on intake, digestibility and rumen digesta dynamics. PhD Thesis, University of Aberdeen, Scotland.

Manyuchi, B., Hovell, F.D. DeB., Ndlovu, L.R., Topps, J.H., Tigere, A., 1996. Feeding napier hay as supplement to sheep given poor quality natural pasture hay: Effects of level of napier hay supplement and inclusion of urea in the basal diet on intake and digestibility. Anim. Feed Sci. Technol. 63, 123–135.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci., Camb. 93, 217–222.

Merry, R.J., Theodorou, M.K., Raurich, M.G., Dhanoa, M.S., 1991. Use of head-space gas pressure in batch cultures to assist in determination of nutritive value of silage to rumen bacteria, In: Forage Conservation Towards 2000 Landbauforschung Volkenrode, pp. 451–454.

Moss, A.R., Givens, D.I., Phipps, R.H., 1992. Digestibility and energy value of combinations of forage mixtures. Anim. Feed Sci. Technol. 39, 151–172.

Prasad, C.S., Wood, C.D., Sampath, K.T., 1994. Use of in vitro gas production to evaluate rumen fermentation of untreated and urea treated finger millet straw (Eleusine coracana) supplemented with different levels of concentrate. J. Sci. Food Agric. 65, 457–464.

Satter, L.D., Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutr. 32, 199–208.

Sampath, K.T., Wood, C.D., Prasad, C.S., 1995. Effect of urea and by-products on the in vitro fermentation of untreated and urea treated finger millet (*Eleusine coracana*) straw. J. Sci. Food Agric. 67, 323–328.

Silva, A.T., Ørskov, E.R., 1988. The effect of five different suuppllements on the degradation of straw in sheep given untreated barley straw. Anim. Feed Sci. Technol. 19, 289–298.

Silva, A.T., Greenhalgh, J.F.D., Ørskov, E.R., 1989. Influence of ammonia treatment and supplementation on the intake, digestibility and weight gain of sheep and cattle on barley straw diets. Anim. Prod. 48, 99–108.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185–197.

Tilley, J.M.A., Terry, R.A., 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassland Soc. 18, 104–111.