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Effect of Urea and By-Products on the In-Vitro Fermentation of Untreated and Urea Treated Finger Millet (*Eleusine coracana*) Straw

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Abstract: The in-vitro fermentation characteristics of untreated and 50 g litre^{-1} urea-treated finger millet (*Eleusine coracana*) straw with four supplements (urea, rice bran, cottonseed and groundnut cakes) at three different ratios of straw: supplement were investigated. Gas production was greater from treated than untreated straw; groundnut cake was the most rapidly fermented supplement followed by cottonseed cake and rice bran. Urea incubated alone inhibited gas production. Untreated and treated straws were incubated with 22, 30 and 37 g rumen degradable nitrogen from the supplements per kg organic matter digested. Significant ($P < 0{\cdot}05$) positive interactive effects on gas production were observed with untreated straw at all three levels of groundnut cake supplementation after 12, 52 and 166 h incubation. Similar interactions were observed for cottonseed cake supplementation of untreated straw and groundnut cake supplementation of treated straw, although statistical significance was not achieved for all supplementation levels at the three times for which data were analysed. No consistent significant interactive effects in gas production were observed between cottonseed cake and treated straw. Rice bran inhibited gas production after 12 h but, subsequently, had little effect on either type of straw. Urea inhibited the gas production from both straws at all three ratios of supplementation. Urea also significantly reduced dry matter disappearance of treated straw at two of three levels of urea supplementation. Interactive effects on gas production were most pronounced in the early stages fermentation and appeared to be related to the high content of highly fermentable material particularly in groundnut cake but also in cottonseed cake.

Key words: supplementation, straw, digestibility, urea treatment, gas production, rumen fermentation

INTRODUCTION

In India and many other tropical countries straw is a major component of ruminant diets. Animal performance on such diets can be poor due to low intakes and digestibilities. The major contributing factors for the low digestibility of straw diets are the unbalanced supply of nutrients due to low nitrogen and high fibre presence of antinutritional factors like silica, 1993). Various methods physical (Oje and Mowat 1978), chemical (Rai and Mudgal 1986), and biological (Rai and Mudgal 1983) treatments have been developed to improve the nutritive value of straw, but each method has its own limitations for practical implementation.

An alternative approach is to provide the nutrients which are deficient in straw as supplements to stimulate the fermentation of fibre by the rumen microbes (Preston and Leng 1987; Schiere *et al* 1989). Urea is a relatively inexpensive source of non-protein nitrogen, groundnut cake and cottonseed cake are by-products available in many tropical countries which can be used as protein supplements, and rice bran is a widely available by-product which could be used with straw. Prasad

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et al (1994) described the use of an in-vitro fermentation technique (Merry *et al* 1991; Theodorou *et al* 1994) to evaluate supplementation of finger millet straw with different proportions of a concentrate mixture. The objective of the present experiment was to study the use of the same technique to compare different sources of nitrogen together with rice bran as supplements to finger millet (*Eleusine coracana*) straw.

EXPERIMENTAL

Materials

Finger millet straw, groundnut cake (GNC), cottonseed cake (CSC) and rice bran were obtained from commercial sources near Bangalore, India. Untreated and 50 g litre^{-1} urea (ammoniated) finger millet straw (treated straw) were prepared from a single batch of starting material and were from the same batch of materials as those used by Prasad *et al* (1991) in a feeding trial with concentrate supplementation. The straw was treated by spraying with 50 g litre^{-1} urea (analytical grade reagent, BDH/Merck Ltd, UK) at the rate of 1 litre urea solution per kg straw. It was stored in pits for 14 days covered by polythene sheets (Prasad *et al* 1991).

Rumen nitrogen degradability of the types of supplements used was previously estimated by incubating similar samples in nylon bags of pore size 40 μm for 3, 6, 9, 15 and 24 h in fistulated cows (Sampath 1990). The effective degradability was calculated by iterative least squares procedure for a fractional outflow of 0.05 per hour (Ørskov and McDonald 1979). The composition, nitrogen degradability values and calculated rumen degradable nitrogen (RDN) of the different supplements and straws are presented in Table 1. The mean proportion of straw/supplement was chosen to give 30 g RD per kg of organic matter digested (OMD). The amou was increased by 25% (to 37 g RDN kg^{-1} OMD) a decreased by 25% (to 22 g RDN kg^{-1} OMD) to gi the three levels used. The quantities of supplement us are given in Table 2. The RDN provided by straw w not taken into account for calculating the total RDN the diet.

Gas production method

Gas production was measured with a pressure tran ducer as described by Merry *et al* (1991) and Theo dorou *et al* (1994) and modified by Prasad *et a* (1994). The volume of gas produced was measured ever 3 h initially, then at lengthening intervals as the rate c production declined. Each fermentation was performe in triplicate for 166 h and the residue recovered by fil tration into preweighed scintered glass crucibles, poro sity P160 (British Standard grade 1), dried and weighed.

Computation of data and statistical analysis

Gas production data were corrected to a per g dry substrate basis. The cumulative gas produced after 12, 52 and 166 h were used for comparison between substrates. The data were analaysed to see if there were statistically significant interactive effects between the straws and the supplements, that is if the gas produced by the mixture was greater than predicted by summing the gas produced by the two substrates individually. Pooled standard errors were calculated for the differences between mean gas production values of the different treatments. Gas production assuming no interactions was predicted by multiplying the weights of straw and substrate by their gas production when fer-

TABLE 1
Composition of straw and supplements used for in-vitro and in-situ trials

	Organic matter (*g kg^{-1} dry matter*)	*Ether extractable material* (*g kg^{-1} dry matter*)	*NDF*[a] (*g kg^{-1} dry matter*)	*Nitrogen* (*g kg^{-1} dry matter*)	*In-situ nitrogen degradability* (%)[b]	*RDN*[c] (*g kg^{-1} dry matter*)
Untreated straw	930	12	780	5	ND[d]	ND
Treated straw	927	10	765	15	ND	10[e]
Groundnut cake	929	89	140	79	70	55
Cottonseed cake	936	18	220	80	50	40
Rice bran	854	11	540	25	45	11
Urea	998	ND	ND	463	100	463

[a] NDF, neutral detergent fibre.
[b] Source: Sampath (1990).
[c] RDN, rumen degradable nitrogen, calculated from nitrogen content and degradability.
[d] ND, not determined.
[e] Nitrogen derived from urea treatment was taken as being 100% degradable.

TABLE 2

Amounts of supplement (g per g substrate[a]) used for in-vitro gas production studies (Supplements used singly, total substrate used was 1 g for each in-vitro replicate)

Supplement	*Level of supplement (RDN[b] per kg OMD[b])*		
	22	*30*	
Supplemented untreated straw			
Groundnut cake	0·18	0·23	0·27
Cottonseed cake	0·23	0·29	0·34
Rice bran	0·52	0·6	0·65
Urea	0·025	0·034	0·04
Supplemented treated straw			
Groundnut cake	0·1	0·17	0·23
Cottonseed cake	0·12	0·22	0·29
Rice bran	0·34	0·51	0·6
Urea	0·012	0·024	0·034

[a] Substrate = supplement plus straw (treated or untreated).
[b] RDN, rumen degradable nitrogen. OMD, organic matter digested.

mented alone. If the predicted levels of gas production fell outside the 95% confidence levels of measured gas produced by supplemented straws these were taken as statistically significant interactive effects ($P < 0·05$). Dry matter disappearance after 166 h incubation was similarly analysed.

RESULTS AND DISCUSSION

Gas production characteristics

The gas production curves for individually fermented straws and supplements are given in Fig 1. The volumes of gas produced after 12, 52 and 166 h for 1 g (dry basis) of the supplements and straws when fermented alone are given in Table 3. Gas production after 12 h incubation is an indicator of initial fermentation, after 52 h an indicator of the extent of fermentation which may be found *in vivo* (Prasad *et al* 1994) and after 166 h an indicator of the end point of fermentation.

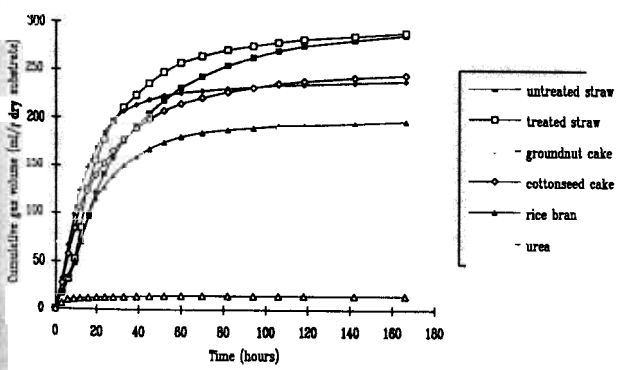

Fig 1. Cumulative gas produced for individual supplements and straws at different times of incubation.

The gas production curves and gas production after 12 h indicated that GNC, in particular, and CSC were fermented relatively quickly, presumably because they contained relatively high amounts of readily fermentable material. This can be inferred from the relatively low neutral detergent fibre contents and is also reflected in the ranking GNC > CSC > rice bran for in-situ nitrogen degradability (see Table 1). The initial gas production rate of rice bran was similar to that of untreated straw. The gas production for urea alone was significantly ($P < 0·05$) lower than that without any added substrate, so urea appeared to be slightly toxic to the microbes in the in-vitro system.

Urea treated straw had a higher gas production than untreated straw for much of the incubation period. Treated straw exhibited a marked lag phase for about 9 h before the rate of gas production accelerated, the lag phase for untreated straw was similar but the subsequent rate of gas production increased relatively little. After 52 h cumulative gas production from treated straw was significantly higher ($P < 0·05$) than that from untreated straw. The final gas productions (166 h incubation) were, however, similar (differences not statistically significant, $P > 0·05$). The in-vivo digestibilities of the untreated and treated straws were 52 and 69%, respectively (Prasad *et al* 1991). These relative digestibilities are reflected in the gas production data after 52 h incubation, but not after 166 h.

Table 3 shows that dry matter disappearances over 166 h fermentations were higher for GNC and CSC than for untreated straw ($P > 0·05$), but the gas production data after 166 h were higher for untreated straw than these two supplements ($P > 0·05$). There are

TABLE 3
In-vitro gas production and dry matter disappearance of straws and supplements

Feed	*Cumulative gas produced in vitro (ml per g dry matter) after different lengths of incubation (h)*			*Dry matter disappearance (% by filtration) after 166 h incubation*
	12	*52*	*166*	
Untreated straw	71	219	286	73·9
Treated straw	85	248	289	84·7
Groundnut cake (GNC)	124	223	239	80·7
Cottonseed cake (CSC)	106	208	245	79 5
Rice bran	71	175	197	53·4
Urea	11	14	15	100·0
No substrate	13	22	25	ND[a]
Standard error	3·4	3·6	4	0·86

[a] ND, not determined.

several factors which may have caused this discrepancy acting individually or in combination with each other. The supplements may have contained a high content of soluble material which was not recovered by filtration but not degraded, hence not contributing to gas production. The stoichiometry of gas production may have been different for the supplements and straws; Menke *et al* (1979) indicated that gas production reflected more the content of digestible carbohydrates than of protein and fat. Changes in the proportions of different volatile fatty acids would also affect the amount of carbon dioxide released from the buffer as the acids were neutralised as well as the amount of carbon dioxide released directly. Substrates with very different compositions may therefore have different in-vitro fermentation characteristics depending on whether they are measured on the basis of their gas production or dry matter disappearance.

Interactive effects between feeds on gas production

Table 4 presents data on the percentage difference between the observed gas production for straws plus supplements compared with that calculated from the gas production from straws and supplements fermented separately. Positive data indicate that gas production for the supplemented straws was higher than that predicted from the separate fermentation of substrates, negative data indicate a lower gas production. Similar data are presented for dry matter disappearance (DMD).

The rapidly fermentable GNC greatly increased the gas production compared with predicted amounts after 12 h when used to supplement untreated and treated straw. The size of the interaction declined considerably as fermentation progressed, but was statistically significant after 166 h incubation for both untreated and treated straws at all three levels of supplementation.

Positive interactive effects were also observed for untreated straw supplemented with CSC, these effects being statistically significant ($P < 0{\cdot}05$) for all three supplementation levels after 52 and 166 h, and for the highest supplementation level after 12 h. In contrast, treated straw supplemented with CSC showed positive interactive effects with the highest level of supplementation (but only achieving statistical significance after 166 h incubation) and negative interactive effects with the lowest supplementation level (but only statistically significant after 12 h incubation).

Rice bran gave rise to negative interactive effects with both untreated and treated straws after 12 h incubation, but this effect rapidly declined to the extent that no statistically significant interactions were observed after 52 h, interactions being generally positive by this time. Urea gave rise to large negative interactions, particularly in the early stages of gas production.

Interactive effects generally reflected the gas production characteristics of the supplements when fermented alone, the higher the gas production after 12 h incubation the more positive the observed interactive effects. The response was largely during the initial stages of fermentation and declined as this proceeded. Gas production appeared to be responding to supplementation with readily fermentable material which presumably acts as a ready energy source, possibly also a source of amino acids. This probably acts by stimulating the activity of the rumen microorganisms which in turn would accelerate the digestion of the straw. Positive interactive effects generally increased with

TABLE 4

Differences (%)[a] between gas production and dry matter disappearance observed for supplemented straw and that predicted from straw and supplement fermented separately

Supplement	*g RDN[b] per kg OMD[b]*	*Incubation periods (h)*			*DMD[c]*
		12	*52*	*166*	
Supplemented untreated straw					
Groundnut cake	37	16·33*	5·24*	3·58*	1·40
	30	14·36*	5·51*	3·69*	1·11
	22	14·51*	6·49*	4·82*	1·30
Cottonseed cake	37	13·23*	5·97*	4·91*	1·71
	30	4·94	3·48*	3·68*	1·42
	22	2·27	5·10*	5·21*	1·48
Rice bran	37	−12·44*	2·28	2·56	2·68
	30	−12·02*	2·05	2·78	1·46
	22	−11·47*	2·26	3·50*	2·47
Urea	37	−53·13*	−15.02*	−7·60*	1·68
	30	−41·94*	−13·39*	−7·67*	0·42
	22	−47·42*	−13·34*	−7·34*	−1.14
Supplemented treated straw					
Groundnut cake	37	15·09*	3·66*	3·39*	−0·57
	30	16·26*	2·92	3·20*	−1·45
	22	11·04*	2·58	3·40*	−2·49
Cottonseed cake	37	6·14	2·53	3·77*	−1·19
	30	−2·40	0·28	1·98	−1·26
	22	−12·64*	−3·63	−1·44	−1·52
Rice bran	37	−6·10	2·34	3·57	−0·64
	30	−9·05*	0·35	1·00	−0·34
	22	−20·74*	−3·12	−1·58	−2·24
Urea	37	−29·61*	−8·16*	−4·89*	−3·90*
	30	−30·48*	−8·14*	−4·71*	−1·02
	22	−31·68*	−8·66*	−5·36*	−3·51*

[a] Difference (%) $= \frac{\text{(observed gas production-predicted gas production)}}{\text{predicted gas production}} \times 100$

[b] RDN, rumen degradable nitrogen. OMD, organic matter digested.

[c] DMD, Dry matter disappearance measured gravimetrically after 166 h incubation. Difference (%) calculated as in footnote *a* with DMD replacing gas production.

* Differences statistically significant ($P < 0.05$).

increasing supplementation as would be expected. *In vivo*, supplementation with readily fermentable material can inhibit the digestion of roughages by reducing the pH of the rumen (Mould *et al* 1983). In the buffered in-vitro system used here the pH is maintained above the pH 6·0–6·1 threshold below which cellulosis is totally inhibited. Also other nutrients such as minerals and nitrogen are available in the in-vitro buffer. These could be limiting *in vivo*, resulting in the depression of fibre digestion by readily degradable material, the so-called carbohydrate effect observed by Mould *et al* (1983).

DMD

The highest and lowest levels of urea supplementation of treated straw produced statistically significant ($P < 0.05$) reductions in DMD, otherwise no signficant interactions were observed. The possible presence of microbial material in the undigested residue would reduce the DMD value which could explain why no significant increases in DMD were observed when there were increases in gas production. Factors which could affect the stoichiometry of gas production may also be relevant (see above). Interactive effects in gas production were also generally low after 166 h incubation, when DMD was assayed, so the lack of significant positive interactive effects on DMD is not necessarily inconsistent with the gas production data. The inhibition of gas production by urea was reflected in reductions in DMD in treated straw supplemented with urea.

In contrast, the substantial reduction in gas production from urea-supplemented untreated straw occurred without producing a consistent effect on

DMD suggesting that there was an increase in the production of microbial material and/or changes in the stoichiometry of gas production.

CONCLUSIONS

From the study it was concluded that supplementing straw based diets with GNC and CSC can increase in-vitro gas production, presumably due to the stimulation of the activity of rumen microbes. The positive interactive effects observed here between some supplements and straw mean that the benefits of in-vivo supplementation may be potentially greater than predictable from the characteristics of the supplements and straws alone. The gas production technique could be a simple and rapid tool for studying the effect of various supplements on the digestion of crop residues and other roughage diets in use in India and elsewhere. It could improve the basis on which rations are formulated. It must, however, be used with some caution as there could be important differences between in-vitro and in-vivo conditions under certain circumstances.

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