In vitro fibre digestion: associative effects in tropical grass-legume mixtures

W.F. BROWN, Z.Q. LAI[1] AND
W.D. PITMAN
Agricultural Research and Education Center, University of Florida, Ona, Florida, U.S.A.

Abstract

This research evaluated the effects of source of ruminal fluid inoculum and grass:legume proportion on the kinetics of *in vitro* neutral detergent fibre (NDF) digestion in grass-legume mixtures. Species used were the grasses, bahiagrass (*Paspalum notatum*) and limpograss (*Hemarthria altissima*), and the legumes, aeschynomene (*Aeschynomene americana*) and hairy indigo (*Indigofera hirsuta*). Sources of ruminal fluid were from steers fed limpograss hay (6.0 g/kg N) alone, or limpograss hay supplemented with urea or cottenseed meal.

In vitro NDF digestion of 100% grass was greater when ruminal fluid from steers fed limpograss hay plus supplemental N was used than ruminal fluid from steers fed limpograss hay only. This suggested that activity of ruminal micro-organisms from steers fed limpograss hay only was limited in N and/or other factors necessary for optimal *in vitro* fibre digestion. When ruminal fluid from steers fed limpograss hay only was used, mixtures of aeschynomene with either grass resulted in *in vitro* NDF digestion values that were greater than the weighted average of *in vitro* NDF digestion values of the individual forages. The magnitude of these increases was similar to the improvement in *in vitro* NDF digestion of pure grass obtained using ruminal fluid from steers fed limpograss hay plus supplemental N, suggesting that aeschynomene provided soluble and/or degradable N to the *in vitro* fermentation. The likely mode of action of the positive associative effects on *in vitro* NDF digestion was a reduced lag time for the initiation of fibre digestion.

Resumen

Se evaluaron los efectos de la fuente del liquido ruminal del inóculo y de la proporción gramínea: leguminosa sobre la cinética de la digestión in vitro *de la fibra detergente neutra (NDF) de las mezclas de gramíneas y leguminosas. Las especies usadas fueron, las gramíneas: Bahiagrass (*Paspalum notatum*) y limpograss (*Hemarthria altissima*), y las leguminosas: aeschynomene (*Aeschynomene americana*) y hairy indigo (*Indigofera hirsuta*). Las fuentes de liquido ruminal fueron obtenidas de novillos alimentados ya sea solo con heno de limpograss (6.0 g/kg N), o con heno de limpograss suplementado con urea y torta de algodón.*

La digestión in vitro *de la NDF de 100% gramínea fue mayor cuando el liquido ruminal fue obtenido de novillos alimentados con heno de limpograss más N suplementario que cuando fue obtenido de novillos alimentados solo con heno de limpograss. Lo anterior sugiere que la actividad ruminal de los micro-organismos de los novillos alimentados solo con heno de limpograss fue limitada por el N y/u otros factores necesarios para una óptima digestión* in vitro *de la fibra. Cuando se utilizó liquido ruminal obtenido de novillos alimentados con solo heno de limpograss, las mezclas de aeschynomene con cualquiera de las gramíneas produjeron valores mayores de digestión* in vitro *de la NDF que el promedio equilibrado de los valores de la digestión* in vitro *de la NDF de los forrajes individualmente. La magnitud de este incremento fue similar al mejoramiento de la digestión* in vitro *de la NDF de la gramínea pura obtenido con el uso de liquido ruminal de los novillos alimentados con limpograss más N suplementario, lo cual sugiere que aeschynomene proporcionó N soluble y/o degradable para la fermentación* in vitro. *El posible modo de acción de los efectos positivos asociados sobre la digestibilidad* in vitro *de la NDF fue mediante la reducción en tiempo del inicio de la digestión de la fibra.*

Correspondence: Dr W.F. Brown, Agricultural Research and Education Center, Route 1 Box 62, Ona FL 33865, USA.
[1]Present address: Guangxi Animal Science Research Institute, Nanning, Guangxi, Peoples Republic of China.

Introduction

Low crude protein concentration and high indigestible fibre content of many tropical grasses have been related to reduced voluntary intake (Milford and Minson 1965) and animal performance (Minson and Milford 1967), resulting in low production levels or costly supplemental feeding. Input costs for providing adequate nutrient supply to ruminants on low-quality forage-based diets might better be directed at improving forage utilization rather than for supplemental feed.

Incorporation of legumes into tropical grass pastures has been used to improve the nutritional status of ruminants above that obtained with grass alone. However, little is known about specific benefits of individual legumes, or ranges in nutritional improvement with legumes of varying quality attributes. In low-protein (less than 70 g/kg crude protein) forage-based diets, the supplementary effect of legume addition has been attributed to the legume overcoming a N deficiency in the forage (McLeod and Minson 1969), by providing ruminally degradable protein (Paterson *et al.* 1981; Brandt and Klopfenstein 1986). In higher-protein (greater than 100 g/kg crude protein) forage-based diets, thought to be adequate in ruminally degradable protein, responses to legume addition have been similar to responses from slowly degraded proteins (Flores *et al.* 1979) suggesting that much of the legume protein may escape ruminal degradation. Variation may exist in the protein degradation characteristics in tropical legumes, because van Eys *et al.* (1986) attributed the positive response to legume supplementation of napier grass (*Pennisetum purpureum*) that was 120 g/kg crude protein to ruminal degradation characteristics of the legume protein.

Because one dietary feed ingredient may influence digestibility of other ingredients, the nutritional value of a mixture of feeds may not always be derived from an evaluation of the individual components. The interaction of one feed ingredient upon the digestibility and utilization of another has been termed an associative effect. Positive associative effects have been observed by tropical legume addition to forage-based diets (Ash 1990), but little information is available describing the mode of action of these effects. The objectives of this research were to investigate associative effects and their mechanism of action on the *in vitro* fibre digestion characteristics in tropical grass-legume mixtures.

Materials and Methods

Herbage samples of the tropical grasses Pensacola bahiagrass (*Paspalum notatum*) and Bigalta limpograss (*Hemarthria altissima*), and the tropical legumes aeschynomene (*Aeschynomene americana*) and hairy indigo (*Indigofera hirsuta*) were harvested from ungrazed pastures in August (mid-summer) 1986. Bahiagrass and limpograss pastures were in a late vegetative growth stage following a regrowth period of 8 wk. Bahiagrass and limpograss samples were clipped to a 5 cm stubble height. Bahiagrass was essentially all leaf. Limpograss consisted of approximately 50% leaf. The aeschynomene and hairy indigo samples consisted of the upper 20 cm of legume stands that were approximately 40 cm in height. These legume samples comprised the leafy, upper canopy which is typically the most readily grazed portion. Aeschynomene consisted of approximately 55% leaf, while hairy indigo was approximately 65% leaf. Grass and legume samples were dried at 50 °C in a forced air drier, ground through a 1 mm screen and stored for further analysis.

Subsamples of the four forages were analyzed for laboratory dry matter (DM), organic matter (OM) and total N concentration according to AOAC (1975). Neutral detergent fibre (NDF) concentration was determined according to Goering and Van Soest (1970). All analyses were conducted in triplicate.

The influence of ruminal fluid inoculum source on *in vitro* NDF digestion characteristics of tropical grass-legume mixtures was evaluated using the following procedures. Six ruminally fistulated steers (approximately 300 kg, *Bos taurus* X *Bos indicus*) were fed the following three diets (two steers per diet) to establish three ruminal fluid inoculum sources: (1) limpograss hay only, (2) limpograss hay plus urea and (3) limpograss hay plus cottonseed meal (CSM). The limpograss hay was harvested in the fall after approximately 14 weeks regrowth, and was stored in round bales (approximately 325 kg). Total N concentration of the hay averaged 6.0 g/kg. Steers were housed individually in 5 m X 10 m outdoor pens, and the limpograss hay was fed in ad libitum quantities in round bale feeders. Steers receiving CSM were fed 0.6 kg DM of CSM daily, while steers receiving urea were fed 0.5 kg DM corn starch plus 0.1 kg DM of urea daily. Corn starch was added to the urea to equalize the energy from CSM. All supplements were completely consumed each day. Steers had free access to water and a commercial mineral mix. Steers were fed the diets for 14 days before ruminal fluid was collected.

In vitro NDF digestion of the following grass-legume combinations were evaluated: bahiagrass-aeschynomene, limpograss-aeschynomene, bahiagrass-hairy indigo and limpograss-hairy indigo. Grass:legume mixtures of the following proportions were evaluated: 100:0, 90:10, 75:25, 50:50, 25:75, 10:90 and 0:100. Ruminal fluid from steers fed the above three diets was also evaluated. Ruminal fluid from each of the two steers fed a given diet was collected approximately 2 h after feeding the supplement, and was mixed on an equal volume basis. This resulted in a 4 X 7 X 3 factorial arrangement of treatments. All *in vitro* NDF digestion analyses were conducted in triplicate within runs, with duplicate runs.

For *in vitro* NDF digestion analysis, an approximate 0.5 g sample of a given grass:legume mixture was incubated with 50 ml of the appropriate ruminal fluid:McDougall's buffer (1:1) solution (McDougall 1948) for 48 h following the first stage of the Tilley and Terry (1963) technique. No supplemental N was added to the inoculum. After 48 h, fermentation was halted by the addition of 2 ml of 50 g/kg $HgCl_2$. Inoculum and residue were then refluxed in NDF solution (Goering and Van Soest 1970). *In vitro* NDF digestion was expressed as percentage ash free, OM basis.

In vitro NDF digestion (Y-value) was regressed against percentage grass in the grass-legume mixture (X-value) using values for the 100:0 and 0:100 grass-legume mixtures. Associative effects measured in units of percentage digestion for the 90:10, 75:25, 50:50, 25:75 and 10:90 grass-legume mixtures, were calculated as the deviation of actual *in vitro* NDF digestion values from the regression line established using values for the 100:0 and 0:100 grass-legume mixtures.

Within an *in vitro* run, a mean value for *in vitro* NDF digestion and associative effect for a given treatment was calculated. Values from the two *in vitro* runs were used to calculate an overall mean and standard error.

Kinetics of *in vitro* NDF digestion in grass-legume mixtures and their associative effects were also measured. Grass-legume combinations of bahiagrass-aeschynomene, limpograss-aeschynomene, bahiagrass-hairy indigo and limpograss-hairy indigo were evaluated. Grass:legume mixtures of the following proportions were evaluated: 100:0, 75:25, 50:50, and 0:100. This resulted in a 4 X 4 factorial arrangement of treatments. *In vitro* lag time, rate and extent of NDF digestion were determined by incubating triplicate tubes in duplicate runs for 0, 4, 8, 12, 24, 36, 48, 72 and 96 h with 50 ml of a ruminal fluid: McDougall's buffer (1:1) solution (McDougall 1948). Ruminal fluid was collected from a ruminally fistulated steer maintained on the same limpograss hay as that described previously. No supplemental N was added to the inoculum. At appropriate times, fermentation was halted by the addition of 2 ml of 50 g/kg $HgCl_2$. The 0 h samples were inoculated, and $HgCl_2$ added immediately. Inoculum and residue were refluxed in NDF solution (Goering and Van Soest 1970) within 1 d after removal from the incubator. Rate of NDF digestion, and lag time were calculated by nonlinear least squares (Mertens and Loften 1980). Extent of NDF digestion was considered to be that occurring after 96 h fermentation. Within an *in vitro* run, a mean value for lag time, rate and extent of *in vitro* NDF digestion and associative effects for a given treatment were calculated. Values from the two *in vitro* runs were used to calculate an overall mean and standard error.

Results

Chemical composition of the individual forages are presented in a companion paper (Brown and Pitman 1991). Total N and NDF concentrations were 13.3 and 730 g/kg for bahiagrass, 6.8 and 762 g/kg for limpograss, 39.2 and 427 g/kg for aeschynomene and 30.4 and 347 g/kg for hairy indigo.

When ruminal fluid from steers fed limpograss hay only was used, *in vitro* NDF digestion of 100% bahiagrass (39.3%) was similar to that of 100% limpograss (38.5%; Table 1). *In vitro* NDF digestion of both grasses was increased when ruminal fluid from steers fed limpograss hay plus supplemental N was used compared to ruminal fluid from steers fed limpograss hay only. However, limpograss showed a greater increase in *in vitro* NDF digestion than did bahiagrass when ruminal fluid from steers fed limpograss hay plus supplemental N was used compared to ruminal fluid from steers fed limpograss hay only.

Table 1. Influence of ruminal fluid inoculum source on *in vitro* neutral detergent fibre digestion and associative effects in tropical grass-aeschynomene mixtures

				Grass-legume proportions							
		Ruminal	% Grass	100	90	75	50	25	10	0	
Grass[1]	Legume[2]	fluid	% Legume	0	10	25	50	75	90	100	s.e.
***In vitro* neutral detergent fibre digestion (%)**											
Bahia	Aeschynomene	Limpo only		39.3	43.0	47.7	49.8	46.9	45.8	44.6	0.75
		Limpo + urea		45.8	49.6	48.3	47.6	47.2	46.3	44.4	
		Limpo + CSM		46.7	49.7	49.0	48.9	48.1	47.0	46.1	
Limpo	Aeschynomene	Limpo only		38.5	47.5	53.3	56.6	51.5	46.1	44.6	
		Limpo + urea		55.2	55.0	56.6	56.1	52.2	48.2	44.4	
		Limpo + CSM		57.1	58.0	57.2	56.9	53.0	49.0	46.1	
Associative effects (percentage units of digestion)											
Bahia	Aeschynomene	Limpo only			3.1	7.1	7.9	3.6	1.7		0.50
		Limpo + urea			3.9	2.9	2.5	2.5	1.8		
		Limpo + CSM			3.1	2.5	2.5	1.9	0.8		
Limpo	Aeschynomene	Limpo only			8.4	13.3	15.1	8.4	2.1		
		Limpo + urea			0.9	4.1	6.3	5.1	2.7		
		Limpo + CSM			2.0	2.9	5.3	4.2	1.8		

[1]Bahia = *Paspalum notatum*, Limpo = *Hemarthria altissima*
[2]Aeschynomene = *Aeschynomene americana*

Table 2. Influence of ruminal fluid inoculum source on *in vitro* neutral detergent fibre digestion and associative effects of tropical grass-hairy indigo mixtures

				Grass-legume proportions							
		Ruminal	% Grass	100	90	75	50	25	10	0	
Grass[1]	Legume[2]	fluid	% Legume	0	10	25	50	75	90	100	s.e.
***In vitro* neutral detergent fibre digestion (%)**											
Bahia	Hairy indigo	Limpo only		39.3	37.5	34.3	27.0	21.6	16.3	12.8	2.09
		Limpo + urea		45.8	43.6	40.5	31.0	22.4	17.0	14.7	
		Limpo + CSM		46.7	44.7	41.8	32.0	23.9	18.6	14.8	
Limpo	Hairy indigo	Limpo only		38.5	36.9	33.6	26.4	20.0	15.5	12.8	
		Limpo + urea		55.2	53.9	48.3	37.1	25.9	19.5	14.7	
		Limpo + CSM		57.1	55.5	49.2	37.4	26.8	20.0	14.8	
Associative effects (percentage units of digestion)											
Bahia	Hairy indigo	Limpo only			0.8	1.6	0.9	2.2	0.8		0.16
		Limpo + urea			1.1	2.9	1.0	1.0	0.6		
		Limpo + CSM			1.0	3.0	0.8	1.2	0.6		
Limpo	Hairy indigo	Limpo only			1.0	1.5	0.7	0.7	0.1		
		Limpo + urea			2.8	3.2	2.2	1.1	0.8		
		Limpo + CSM			2.6	2.7	1.5	1.4	1.0		

[1]Bahia = *Paspalum notatum*, Limpo = *Hemarthria altissima*
[2]Hairy Indigo = *Indigofera hirsuta*

In vitro NDF digestion of 100% bahiagrass or 100% limpograss was similar when ruminal fluid from steers fed limpograss hay plus urea or limpograss hay plus CSM was used. *In vitro* NDF digestion of 100% aeschynomene was not influenced by source of ruminal fluid.

Mixtures of aeschynomene with bahiagrass or limpograss resulted in *in vitro* NDF digestion values that were greater than the weighted average of values for the individual forages (Table 1). The magnitude of these positive associative effects were greatest when evaluated with ruminal fluid from steers fed limpograss hay only, and were greater for limpograss than for bahiagrass. When ruminal fluid from steers fed limpograss hay only was used, positive associative effects for *in vitro* NDF digestion of the 75:25 and 50:50 grass-aeschynomene mixtures were similar to the improvement in *in vitro* NDF digestion of pure grass obtained by using ruminal fluid from steers fed limpograss hay plus supplemental N. When ruminal fluid from steers fed limpograss hay plus supplemental N was used, aeschynomene addition to bahiagrass or limpograss resulted in only small positive associative effects on *in vitro* NDF digestion.

In vitro NDF digestion of hairy indigo was low, and not influenced by source of ruminal fluid (Table 2). No associative effects on *in vitro* NDF digestion existed between hairy indigo and bahiagrass or limpograss.

Lag time for the initiation of *in vitro* NDF digestion was greatest for hairy indigo, least for aeschynomene, and similar between bahiagrass and limpograss (Table 3). Mixtures of aeschynomene with bahiagrass or limpograss resulted in a shorter lag time than that calculated from a weighted average of values for the individual forages. Lag time associative effects were greater for limpograss-aeschynomene mixtures than for bahiagrass-aeschynomene mixtures. No associative effects were observed for lag time in mixtures of bahiagrass or limpograss with hairy indigo.

In vitro rate of NDF digestion was greatest for aeschynomene and least for hairy indigo (Table 3). No associative effects were observed for rate of NDF digestion in grass-legume mixtures.

Extent (96 h) of *in vitro* NDF digestion was greater for limpograss than for bahiagrass, and was greater for aeschynomene than for hairy indigo (Table 4). No associative effects were observed for extent of *in vitro* NDF digestion in grass-legume mixtures.

Discussion

In vitro NDF digestion of pure grass was increased by using ruminal fluid from steers fed limpograss hay plus supplemental N in the form of soluble protein or urea compared to ruminal fluid from steers fed limpograss hay only. This suggests that ruminal fluid from steers fed limpograss hay only was limited in N and perhaps other factors necessary for optimal *in vitro* fibre digestion. Improved *in vitro* (el-Shazly *et al.* 1961) and *in vivo* (Van Gylswyk 1970) cellulose digestion was observed by urea supplementation of low-protein forage-based diets. Inclusion of natural proteins to diets containing urea increased bacterial growth response above that obtained with urea alone (Hespell and Bryant 1979). Corn starch added to the urea to equalize energy from CSM may have stimulated ruminal microbial activity above that which would have been

Table 3. Lag time and rate of *in vitro* neutral detergent fibre digestion and associative effects in tropical grass-legume mixtures

			Grass-legume proportions				
		% Grass	100	75	50	0	
Grass[1]	Legume[2]	% Legume	0	25	50	100	s.e.
Lag time (h)							
Bahia	Aeschynomene		14.1	6.7	6.1	5.9	1.25
Limpo	Aeschynomene		13.8	2.0	2.7	5.9	
Bahia	Hairy Indigo		14.1	13.9	15.2	17.3	
Limpo	Hairy Indigo		13.8	13.3	15.3	17.3	
Associative effects for lag time (h)							
Bahia	Aeschynomene			−5.4	−3.9		1.25
Limpo	Aeschynomene			−9.8	−7.2		
Bahia	Hairy Indigo			−1.0	−0.5		
Limpo	Hairy Indigo			−1.4	−0.3		
Rate of digestion (%/h)							
Bahia	Aeschynomene		4.6	4.4	4.8	4.9	0.12
Limpo	Aeschynomene		4.1	4.1	4.5	4.9	
Bahia	Hairy Indigo		4.6	4.3	4.0	3.4	
Limpo	Hairy Indigo		4.1	4.0	3.8	3.4	
Associative effects for rate of digestion (%/h)							
Bahia	Aeschynomene			−0.3	0.0		0.04
Limpo	Aeschynomene			−0.2	0.0		
Bahia	Hairy Indigo			0.0	0.0		
Limpo	Hairy Indigo			0.1	0.0		

[1]Bahia = *Paspalum notatum*, Limpo = *Hemarthria altissima*
[2]Aeschynomene = *Aeschynomene americana*, Hairy Indigo = *Indigofera hirsuta*

Table 4. Extent (96 h) of *in vitro* neutral detergent fibre digestion and associative effects in tropical grass-legume mixtures

			Grass-legume proportions				
		% Grass	100	75	50	0	
Grass[1]	Legume[2]	% Legume	0	25	50	100	s.e.
Extent of NDF digestion (%)							
Bahia	Aeschynomene		65.0	62.4	60.8	55.4	1.97
Limpo	Aeschynomene		71.6	69.3	65.7	55.4	
Bahia	Hairy Indigo		65.0	57.9	53.4	33.9	
Limpo	Hairy Indigo		71.6	63.3	57.5	33.9	
Associative effects for extent of NDF digestion (%)							
Bahia	Aeschynomene			−0.2	0.6		0.61
Limpo	Aeschynomene			1.8	2.2		
Bahia	Hairy Indigo			0.7	4.0		
Limpo	Hairy Indigo			1.1	4.8		

[1]Bahia = *Paspalum notatum*, Limpo = *Hemarthria altissima*
[2]Aeschynomene = *Aeschynomene americana*, Hairy Indigo = *Indigofera hirsuta*

obtained from urea supplementation alone, thereby reducing potential differences in ruminal fluid activity between urea and CSM supplementation.

Associative effects for *in vitro* NDF digestion in grass-aeschynomene mixtures were not found when ruminal fluid from steers fed limpograss hay plus supplemental N was used. However, when ruminal fluid from steers fed limpograss hay only was used, positive associative effects for *in vitro* NDF digestion in grass-aeschynomene mixtures were similar to the improvement in *in vitro* NDF digestion of pure grass obtained by using ruminal fluid from steers fed limpograss hay plus supplemental N. These results suggest that aeschynomene provided soluble and/or degradable N to the *in vitro* fermentation which was similar to the enrichment of ruminal fluid obtained by feeding donor animals supplemental N in the form of urea or CSM. Brown and Pitman (1991) found the N in aeschynomene to be very soluble and likely to be degraded to a large degree in the rumen. Aeschynomene addition may have stimulated microbial growth resulting in greater digestion of grass fibre. Positive effects with other tropical (van Eys *et al.* 1986) and temperate (Paterson *et al.* 1981) legumes have been attributed to the proprotion of ruminally degradable protein in the legume and its effect on microbial protein production. McLeod and Minson (1969) concluded that the supplementary effect of legume addition on *in vivo* DM digestibility of pangola grass (*Digitaria decumbens*) was caused by the legume overcoming a N deficiency in the grass. However in that study, no associative effects were observed for *in vitro* DM digestion of grass-legume mixtures because ruminal fluid from a donor animal fed a grass-legume diet was used. It was suggested that this ruminal fluid supplied sufficient N to overcome the N deficiency in the pangola grass. This may explain why in our study no associative effects for *in vitro* NDF digestion in grass-aeschynomene mixtures were found when ruminal fluid from steers fed supplemental N was used.

Positive associative effects for *in vitro* NDF digestion characteristics were greater in limpograss-aeschynomene than in bahiagrass-aeschynomene mixtures. Brown and Pitman (1991) found that limpograss provided a small pool of ruminally soluble and degradable N leading to low *in vitro* ruminal ammonia release particularly early in the fermentation. However, limpograss had a greater extent of *in vitro* NDF digestion than bahiagrass, resulting in a greater potential for improvement by associative action when supplemental N was provided. Brandt and Klopfenstein (1986) found a greater positive associative effect on daily gain and feed efficiency by alfalfa addition to higher-quality ammoniated, compared to lower-quality nontreated corn cobs. They concluded that positive associative action of forage combinations including legumes may be greater as the companion forage is potentially more digestible.

No associative effects on *in vitro* NDF digestion were observed in grass-hairy indigo mixtures. Brown and Pitman (1991) found *in vitro* ruminal ammonia release from hairy indigo to be less than that from aeschynomene or bahiagrass. Some *Indigofera* species contain anti-quality components (Minson and Hegarty 1984) including high tannin levels (Ahn *et al.* 1989) which may restrict microbial activity leading to reduced fibre utilization.

Positive associative effects were found in lag time for the initiation of *in vitro* NDF digestion in grass-aeschynomene mixtures. Brown and Pitman (1991) found that early in the *in vitro* fermentation, ruminal ammonia release from aeschynomene was at least twice that from bahiagrass or limpograss. Low ruminal ammonia release early in the *in vitro* fermentation from these grasses may have been supplemented by soluble and/or degradable N from aeschynomene resulting in positive associative effects on lag time for the initiation of fibre digestion. The lack of positive associative effects for lag time in grass-hairy indigo mixtures was consistent with low *in vitro* ruminal ammonia release from hairy indigo as observed by Brown and Pitman (1991), perhaps due to anti-quality components as described above.

Positive associative effects were found for 48 h *in vitro* NDF digestion (Table 1), but not for 96 h extent of *in vitro* NDF digestion (Table 4) in grass-aeschynomene mixtures. Positive associative effects for *in vitro* NDF digestion in grass-aeschynomene mixtures measured after 48 h of fermentation may have been due to aeschynomene providing soluble and/or degradable N early in the fermentation resulting in reduced lag time for initiation of fibre digestion. As fermentation time was increased to 96 h, microbial growth and activity may have increased in the *in vitro* tubes containing 100% grass, allowing potential *in vitro* NDF digestion to be expressed. Due to turnover time of ruminal contents, potential extent of fibre digestion in pure grass or grass-legume mixtures is not likely realized. In fact, diets containing legumes typically have a faster rate of passage than pure grass diets (Thornton and Minson 1973), making comparisons of *in vitro* NDF digestion in pure grass, pure legume and grass-legume mixtures early in the fermentation more appropriate. Low *in vitro* NDF digestion of hairy indigo measured at 48 or 96 h was consistent with lower than expected N degradation characteristics and *in vitro* ruminal ammonia release (Brown and Pitman 1991) from hairy indigo.

In conclusion, positive associative effects on *in vitro* NDF digestion occurred when aeschynomene was combined with low N concentration mature grasses. Magnitude of the associative effects was similar to the increase in *in vitro* NDF digestion of pure grass when ruminal fluid from a steer fed low quality hay plus supplemental N was used, suggesting that aeschynomene provided soluble and/or degradable N to the *in vitro* fermentation. Mode of action of the positive associative effect was likely a reduced lag time for the initiation of fibre digestion in grass-aeschynomene mixtures.

Acknowledgements

This paper is published as Florida Agricultural Experiment Station Journal Series number R-01041.

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(Received for publication October 13, 1990; accepted July 20, 1991)