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Availability to ruminants of nitrogen in senesced C_4 tropical grasses

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ABSTRACT

Context. Nutritional standards usually assume that ~10% of the total nitrogen (TN) in forages is indigestible and hence not available to the ruminant. Senesced tropical C_4 grasses often contain TN concentrations that are marginal or deficient to meet the nutrient requirements of ruminants, and low TN availability will exacerbate N deficiencies. **Aim.** The aim of the study was to estimate the availability (i.e. digestibility) to ruminants of TN in mature and senesced C_4 grasses, using data derived from previous experiments and published data. **Methods.** In Dataset 1, forages grown in subtropical or tropical environments, including C_4 (n = 143) and C_3 (n = 15) grasses, were analysed for parameters including TN, acid detergent fibre (ADF), and N insoluble in ADF solution (ADIN). ADIN was used as a measure of unavailable TN. The Dataset 2 analysis included published measurements of TN and ADIN in C_4 (n = 187) and C_3 (n = 45) grasses. **Key results.** In Dataset 1, TN averaged 9.7 and ADIN 1.45 g N/kg diet DM in C_4 grasses. ADIN concentration could be predicted from TN and ADF concentrations by multiple regression ($R^2 = 0.50$; $P < 0.001$). The ratio ADIN/TN averaged 170 g/kg and increased exponentially with increasing ADF concentration ($R^2 = 0.43$; $P < 0.001$). Also, ADIN/TN was inversely related to TN concentration and DM digestibility. In C_4 grasses containing >400 and >500 g ADF/kg DM, ADIN/TN averaged 190 and 230 g/kg, respectively, and in those containing <10 g TN/kg DM, ADIN/TN averaged 194 g/kg. In these low-quality C_4 grasses only ~80%, and as little as ~50%, of TN was available to ruminants. Dataset 2 produced similar results, although C_4 grass forages were generally of higher quality, most having been harvested during vegetative growth. ADIN/TN was much lower in C_3 grasses (89 g/kg). **Conclusions.** In senesced, low-quality C_4 grasses containing >400 g ADF, <10 g TN or <530 g digestible DM/kg, the proportion of TN available to ruminants is substantially lower than that assumed for forages in general. **Implications.** Low availability of TN in many senesced C_4 grasses needs to be considered when evaluating the adequacy of dietary TN for ruminants grazing senesced tropical grass pastures.

Keywords: acid detergent insoluble N, C_4 grasses, forage digestibility, forage N, low quality forages, N-deficiency, ruminants, tropical forages.

Introduction

There has been extensive investigation of ruminant digestion of the total nitrogen (TN) fractions in forages to understand their breakdown and use as substrates by rumen microbes and, following passage of some forage N to the post-ruminal tract, whether various fractions are digested or are excreted in faeces (Webster 1987; AFRC Technical Committee on Responses to Nutrients 1992). This has often been investigated through use of surgically prepared animals to partition the sites of digestion, and synthetic fibre bags to measure digestion in the rumen and post-ruminally. A laboratory approach to estimating the amounts and proportions of the TN fractions in forages has been used to determine the solubility of TN under various specified conditions (Sniffen *et al.* 1992; Licitra *et al.* 1996). There is consensus that the forage N that is insoluble in acid detergent fibre (ADF) solution (i.e. ADIN) represents the TN that is not digested in the rumen or post-ruminally, and hence provides a reliable measure of the TN that is indigestible and not available to the ruminant (Van Soest 1982; Broderick 1994). It is generally agreed that ~90% of the TN consumed in forages is digested and thus available in ruminants (Webster *et al.* 1982; AFRC Technical Committee on Responses to Nutrients 1992; Sniffen *et al.* 1992). These approaches have, with some variations, been incorporated into evaluations of the nutritional value of feeds and feeding standards (AFRC Technical Committee on Responses to Nutrients 1992; CSIRO 2007; National Academies of Sciences, Engineering and Medicine 2016).

Investigation of the digestion of forage TN fractions has focused on improved and sown species of grasses and legumes during vegetative growth, or soon after physiological maturity, when forages are usually grazed or harvested in higher input ruminant production systems (Wilson and Strachan 1981; Waters *et al.* 1992; Wales *et al.* 1999; National Academies of Sciences, Engineering and Medicine 2016). Relatively little information is available on the digestion of TN fractions in mature, senesced and weathered grasses, particularly C_4 grasses, that usually have low TN concentrations and are highly fibrous. Nevertheless, this is important in some low-input rangeland systems, and crop–livestock systems in low-income countries, where ruminants are highly dependent on senesced C_4 grasses and stovers of low nutritional value as feeds during some seasons of the annual cycle. Also, there is evidence that the N availability in at least some C_4 grass hays is lower than in C_3 grass hays of comparable low nutritional value (Hogan *et al.* 1989; Hogan 1996). Because senesced forages, particularly C_4 grasses, are usually marginal or deficient in TN for ruminants, it is important to understand the availability of this TN to the ruminant.

The present study examined the concentrations of TN, N insoluble in neutral detergent fibre (NDF) solution (i.e. NDIN), ADIN, and fibre components in C_4 grasses, as well as in some C_3 grasses and legumes, grown in subtropical or tropical environments of northern Australia. Forages sampled from a wide range of seasons and sites were used to examine the hypothesis that a substantial proportion of the TN in low-quality tropical C_4 grasses is present as indigestible ADIN. In addition, the study collated published measurements of TN and ADIN in C_4 grasses grown in the subtropics or tropics.

Materials and methods

Dataset 1: forages analysed for N fractions

The forages analysed (n = 194) were obtained from previous experiments. Most (n = 184) had been fed to cattle in individual pens during the development of faecal NIRS calibration equations (Coates and Dixon 2011) or in other, later pen experiments (Kennedy and Charmley 2012; McLennan 2014; RM Dixon and DB Coates, unpublished data) where both the forage and faeces were sampled. The forages were all mechanically harvested from pastures growing in the subtropics or tropics. The forages were considered as classes comprising native or improved species of C_4 grasses, improved species of C_3 grasses, tropical legumes and temperate legumes (Table 1). Subclasses were forage species represented by ≥10 samples, or combinations of several species when there were fewer samples. The native species of C_4 grass forages were generally cut with a forage harvester from swards of native pastures and classified into three subclasses: (1) dominated by *Heteropogon contortus* (black speargrass), (2) dominated by *Astrebla* spp. (Mitchell grasses), or (3) 'mixed native C_4 grasses'. The mixtures of species included *H. contortus*, *Bothriochloa pertusa* (Indian couch) and *Chrysopogon fallax* (golden beard grass), and sometimes *Urochloa mosambicensis* that had invaded native grass paddocks. Hays of the introduced, sown C_4 grass species *Cenchrus ciliaris* and *Chloris gayana* were obtained from commercial farms, and the *U. mosambicensis* was harvested from swards dominated by this species. The subclass 'Other improved C_4 grasses' combined such grasses where there were <10 samples of a species; these included *Pennisetum* spp. (millets), *Setaria* spp., *Brachiaria mutica* (para grass), *Cynodon dactylon* (couch) and *Stenotaphrum secundatum* (buffalo couch). The C_3 grass forages were the cereal crop species *Avena sativa* (oats, n = 6), *Triticum* spp. (wheat, n = 3) and *Hordeum vulgare* (barley; n = 1), all in the vegetative growth stage. The tropical legume *Stylosanthes* included both *Stylosanthes scabra* and *S. hamata*, and 'Other herbaceous legumes' included *Lablab purpureus* (dolichos), *Neonotonia wightii* (glycine), *Centrosema pascuorum* (cavalcade), *Clitoria ternatea* (butterfly pea), *Macroptilium bracteatum* (burgundy bean) and *Arachis pintoi* (peanut). The temperate legume was *Medicago sativa* (lucerne). The C_3 grasses and the legumes had been cut as hays on commercial farms.

The forage samples were analysed for TN by Kjeldahl analyses. NDF and ADF contents, and NDIN and ADIN, were analysed as described by Pichard and van Soest (1977) and Licitra *et al.* (1996). The dry matter digestibility (DMD) of the forages was measured by near-infrared reflectance spectroscopy of faeces (F.NIRS) as described by Coates and Dixon (2011).

Dataset 2: collation of published results reporting ADIN content of C_4 grasses

Data were collated from published papers reporting measurements of the TN and ADIN fractions in C_4 grasses, or mixtures containing predominantly C_4 grasses (n = 187), grown in the subtropics or tropics. Where a study also reported measurements in C_3 grasses (n = 45) or tropical herbaceous legumes expected not to contain tannins

Table 1. Dataset 1: composition (mean, s.d. in parentheses) of the forages (n = 194) in the sample set (subclass) of each species, or group when there were <10 samples of a species.

Pasture species class/subclass	*n*	TN	NDF	NDIN (g/kg DM)	ADF	ADIN	NDIN/TN (g/kg)	ADIN/TN	*n*	DMD (g/kg)
C_4 grasses, native species										
Heteropogon contortus (speargrass)	16	6.6g	749a	3.52ef	508a	1.12d	504ab	175bc	16	496f
Astrebla spp. (Mitchell grasses)	10	8.4fg	715abc	3.33def	479ab	1.43bcd	382cd	166bcd	8	518cef
Other native C_4 grasses	22	6.6g	737ab	3.43f	502a	1.51bcd	520a	243a	22	494f
C_4 grasses, improved species										
Cenchrus ciliaris (buffel)	24	8.8fg	751a	4.63cdef	495ab	1.37cd	522a	169bc	24	521de
Chloris gayana (Rhodes)	24	13.4de	706bc	6.47bc	406e	1.29d	491ab	117de	24	552b
Urochloa mosambicensis	16	10.5efg	732ab	5.92bcd	462bcd	2.08ab	537a	204ab	15	546bc
Other improved C4 grasses	31	11.4def	693c	5.33bcde	437cd	1.45cd	449bc	141cd	30	542bc
C_3 grasses, improved species	15	28.0b	596d	10.85a	359f	2.17ab	387c	89ef	12	630a
Tropical legume species										
Stylosanthes spp.	10	15.5d	594d	4.44cdef	477abc	2.38a	293d	160bcd	10	533bcd
Other herbaceous legumes	15	22.0c	517e	4.19def	430de	2.31a	195e	111def	13	559b
Temperate legumes										
Medicago sativa (lucerne)	11	38.6a	378f	7.50b	277g	2.06abc	185e	52f	9.7	653a
Probability	–	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	–	$P < 0.001$
Average s.e.m.	–	1.63	15.32	0.848	14.1	0.250	29.0	19.5	–	10

Within columns, means followed by the same letter are not significantly different ($P > 0.05$). The constituents measured were total N (TN), neutral detergent fibre (NDF), N insoluble in NDF solution (NDIN), acid detergent fibre (ADF), N insoluble in ADF solution (ADIN), and ratios of these to total N (NDIN/TN and ADIN/TN). Dry matter digestibility (DMD) (n = 183) was measured by using near infrared reflectance spectroscopy of faeces.

(n = 51) and grown in the same environment, those results were included. Measurements of wheat or rice straws as senesced C_3 grasses in the subtropics were also included for comparison. One additional study with C_4 grasses (Buckner *et al.* 2013), in which 'total tract indigestible protein' was measured using disappearance from synthetic fibre bags incubated in the rumen and during passage through the post-ruminal gastrointestinal tract, was also considered. Some of the papers also reported ADF, NDF and NDIN concentrations (105, 120 and 77 of the 187 C_4 grass forage samples, respectively). Samples from treatments within experiments where the pasture had been fertilised with N were excluded. The papers had been published by research groups in North America (n = 11), South America (n = 4), India (n = 7), Africa (n = 4), and elsewhere (n = 5), and often the C_4 grass forages had been measured at various maturity stages during the summer growing season. Genera most often reported and the number of samples were *Cynodon* (31), *Cenchrus* (21), *Panicum* (20), *Andropogon* (16), *Digitaria* (12), *Pennisetum* (11) *Brachiaria*, (9), *Zea mays* (8), *Eragrostis* (7), *Paspalum* (8), *Setaria* (5) and *Sorghum* (5). The dataset included one experiment where *C. dactylon* pasture had been 'stockpiled' after physiological maturity and then sampled several times over 3 months (Scarbrough *et al.* 2002), and another experiment with hay made from mature *Andropogon* spp. ('bluestem range mixed forage') (Hannah *et al.* 1991). One paper reported the changes in two C_4 and two C_3 grass species in the same environment through the summer growth period (Mitchell *et al.* 1997). Another study reported measurements in a range of grass species during the dry season in a southern African semi-arid environment (Gemeda and Hassen 2014). Nine papers included herbaceous tropical legumes (23 species and 51 samples). The forages were considered as groups comprising C_4 grasses, C_3 grasses, tropical legumes and temperate legumes.

Calculations and statistical analyses

Each class of forage within each dataset was divided into six categories of ADIN concentration (<1.00, 1.00–1.49, 1.50–1.99, 2.00–2.99, 3.00–3.99 and >4.00 g/kg DM) and six categories of ADIN/TN (<100, 100–149, 150–199, 200–299, 300–399 and >400 g/kg). In Dataset 1, the attributes of the various subclasses of forages were compared by one way analysis of variance. The relationships between the variables were examined by regression. The statistical package used was Genstat Release 16.1 (VSN International, Hemel Hempstead, UK).

Results

Dataset 1: measured concentrations of N fractions and DMD

The measured forage attributes generally did not differ ($P > 0.05$) among subclasses within the C_4 native and C_4 introduced grass species, or between these classes, and there was no consistent pattern (Table 1). Overall, the C_4 grasses ($n = 143$) averaged 9.7 g TN/kg DM, 1.45 g ADIN/kg DM, 170 g ADIN/kg TN, and ($n = 139$) 526 g digestible DM/kg, but each of these attributes had large variation and range. The C_3 grass and both legume classes were generally much higher in TN and ADIN concentrations, but they had lower ADIN/TN ratios, than the C_4 grasses. However, due to the small number of samples and few species, the C_3 grasses and temperate legumes did not broadly represent these classes of forages. The tropical legumes had high concentrations of TN and ADIN. The distributions of the categories of ADIN and ADIN/TN ratio in the forage classes in Dataset 1 are shown in Fig. 1. Among all C_4 grasses, 44% contained ≥1.50 g ADIN/kg DM and 62% had a ratio of ≥150 g ADIN/kg TN; among C_4 grasses with >400 g ADF/kg DM, 46% contained ≥1.50 g ADIN/kg DM and 74% had a ratio of ≥150 g ADIN/kg TN.

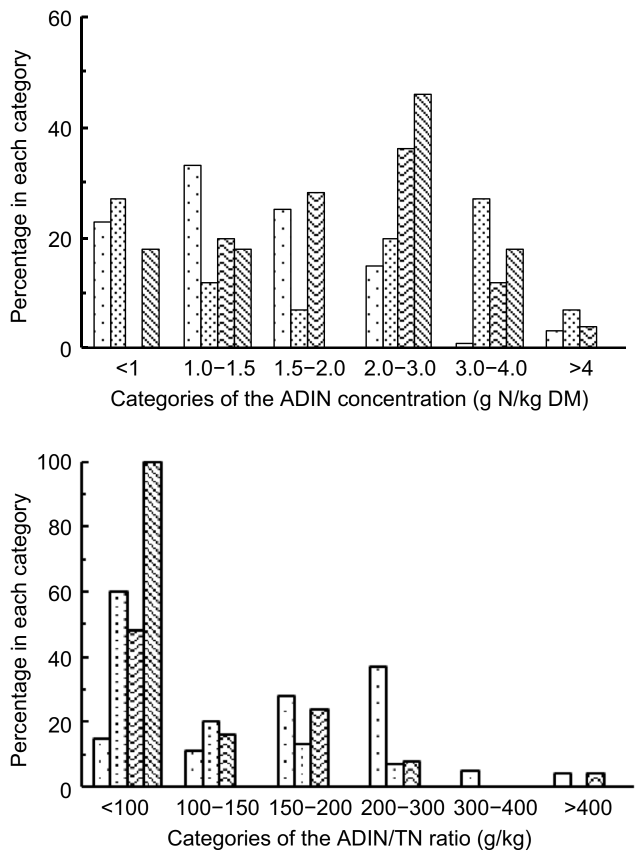

Fig. 1. Dataset 1: distribution of samples of forage classes (left to right): C_4 grasses ($n = 143$), C_3 grasses ($n = 15$), herbaceous tropical legumes ($n = 25$), and temperate legumes ($n = 11$) across categories of ADIN concentration, and categories of ADIN/TN ratio.

Many of the measured forage attributes were correlated ($P < 0.01$), but the correlation coefficients (r) were generally <0.7 and did not allow useful prediction of one variable from another (Table 2). TN was negatively correlated with NDF and ADF ($r = -0.81$ and -0.77), and was positively correlated with DMD ($r = 0.85$). ADIN concentration was not correlated with either NDF or ADF content (both $P > 0.05$), and was only poorly correlated with TN and NDIN ($r = 0.42$ and 0.65, respectively).

Among the C_4 grass forages, the ADIN/TN ratio increased exponentially with increasing ADF content (Fig. 2*a*). Forages containing <400, 400–450, 450–500 and >500 g ADF/kg DM had, on average, ratios of 86, 132, 175 and 230 g ADIN/kg TN, respectively (Table 3). Conversely, increasing TN concentration or DMD was associated with decreasing ADIN/TN ratio (Fig. 2*b*, *c*). In forages containing <5 g TN/kg DM, the ADIN/TN ratio averaged 257 (s.d. 109) g/kg, and in forages containing 5–10 g TN/kg DM, the ADIN/TN ratio averaged 173 (s.d. 82) g/kg (Table 3). As the TN concentration in forages increased to 20 g TN/kg DM, the ADIN/TN ratio decreased to 154 and then 76 g/kg. There was very large variation in the ADIN/TN ratio within each of these categories, especially at low forage TN concentrations. Similar large variation and changes between categories were also observed for four categories of DMD (<500, 500–530, 530–560 and >560 g/kg) (Table 3). In summary, these relationships indicated that the ADIN/TN ratio was increasing, and thus availability of the TN to the ruminant was decreasing, with decreasing nutritional value of the C_4 grass forages. ADIN (g/kg DM) and ADIN/TN ratio (g/kg) could be estimated using multiple regression models:

$$\text{ADIN} = -5.30 + 0.197\text{TN} - 0.00181(\text{TN})^2 + 0.0108\text{ADF}$$
$$(R^2 = 0.50; \text{s.e.} = 0.766, \text{ all coefficients } P < 0.001)$$

and:

$$\text{ADIN/TN} = 222 - 1.29\text{ADF} + 0.00246(\text{ADF})^2$$
$$(R^2 = 0.53; \text{s.e.} = 61.8, \text{ constant } P < 0.01, \text{ coefficients of independent variables } P < 0.001)$$

Dataset 2: collation of published results

The distributions of the categories of ADIN concentration and ADIN/TN ratio in the various forage classes of Dataset 2 are shown in Fig. 3, and the ADIN concentrations and ADIN/TN ratios in Table 3. On average, the C_4 grasses ($n = 187$) contained 1.93 g ADIN/kg DM and had a ratio of

Table 2. Dataset 1: correlation coefficients between the measured variables and ratios from all four classes of forages (n = 194), as well as dry matter digestibility (DMD) and faecal N concentration (n = 141).

	TN	NDF	ADF	NDIN	ADIN	NDIN/TN	ADIN/TN	ADIN/NDIN	DMD
TN	–								
NDF	−0.81**								
ADF	−0.77**	0.78**							
NDIN	0.68**	−0.20**	−0.43**						
ADIN	0.42**	−0.11$^{n.s.}$	0.02$^{n.s.}$	0.65**					
NDIN/TN	−0.41**	0.74**	0.42**	0.30**	0.19**				
ADIN/TN	−0.50**	0.57**	0.71**	−0.20**	0.32**	0.32**			
ADIN/NDIN	−0.28**	0.12$^{n.s.}$	0.52**	−0.40**	0.27**	−0.19**	0.74**		
DMD	0.85**	−0.69**	−0.80**	0.58**	0.16**	−0.29**	−0.61**	−0.47**	
Faecal N	0.82**	−0.63**	−0.68**	0.51**	0.22**	−0.24**	−0.55**	0.42**	0.80**

**$P < 0.01$; n.s., not significant ($P > 0.05$). Measured variables: TN, total N; NDF, neutral detergent fibre; ADF, acid detergent fibre (ADF); NDIN, N insoluble in NDF solution; ADIN, N insoluble in ADF solution.

147 g ADIN/kg TN. The more fibrous grasses with >400 g ADF/kg DM contained, on average, 1.78 g ADIN/kg DM and had a ratio of 189 g ADIN/kg TN, with 55% recording a ratio of ≥150 g ADIN/kg TN. The C_4 grasses containing <400 g ADF/kg DM averaged 1.53 g ADIN/kg DM and 123 g ADIN/kg TN. By comparison, 43% of C_4 grasses with <10 g N/kg DM had a ratio of ≥150 g ADIN/kg TN. In the entire sample set, the concentrations of ADIN were comparable in C_4 grasses, C_3 grasses and tropical legumes (1.93, 1.78 and 2.63 g ADIN/kg DM, respectively), but because the TN concentration was usually lower in the C_4 grasses, the proportion of forages with ratios ≥150 g ADIN/kg TN was higher among C_4 grasses (41%) than C_3 grasses (19%) or tropical legumes (17%). In summary, the profiles of forage constituents in this dataset derived from published measurements by many research groups showed concentrations and trends similar to those observed in Dataset 1.

Discussion

Concentrations and proportions of ADIN as indigestible N

There is consensus that, in grass and herbaceous legume forages, ADIN represents the N that is bound to plant cell walls, is indigestible and not available to the ruminant, and that it typically comprises ~10% of the TN (Van Soest 1982; Webster *et al.* 1988; AFRC Technical Committee on Responses to Nutrients 1992; Broderick 1994). Dataset 1 in the present study clearly showed that in low-quality, senesced C_4 grasses the ADIN/TN ratio was substantial, averaging 190 g ADIN/kg TN in those with >400 g ADF/kg, and similar ratios in those with ≤10 g TN/kg or ≤560 g digestible DM/kg. Importantly, the ADIN/TN ratio in these groups was highly variable, ranging from negligible up to 495 g ADIN/kg TN, and was more closely correlated with ADF than with TN or DMD (Table 2). This is in agreement with associations between ADIN concentration and ADF content reported by Webster *et al.* (1982), AFRC Technical Committee on Responses to Nutrients (1992) and Waters *et al.* (1992). The efficacy of low TN concentrations or low DMD for identifying samples with high dietary ADIN/TN ratio was likely due primarily to the associations among these attributes.

The samples collated from published results as Dataset 2 (Table 3) generally included higher quality C_4 grass forages than Dataset 1 (Table 3). This was most obviously because the forages in Dataset 2 were largely harvested during their vegetative growth rather than after senescence. However, among the C_4 grasses in Dataset 2 that contained >400 g ADF/kg or <10 g TN/kg, the ADIN/TN ratio was comparable to the same classes of C_4 grasses in Dataset 1.

The results are in accord with other evidence of low rumen availability of the TN in some low-quality C_4 grasses, from observations of expectedly low rumen ammonia concentrations in sheep fed such grasses (Hogan *et al.* 1989; Hogan 1996). In summary, there is considerable evidence to support the hypothesis that a substantial proportion of the TN in low-quality C_4 grasses is present as ADIN and therefore indigestible in ruminants.

Variation in ADIN concentration and ADIN/TN ratio among the forages

From the present study and other published studies, it is not possible to separate the effects of factors such as genotype, growth environment and stage of growth of the grasses on ADIN concentration or ADIN/TN ratio. Both Blasi *et al.* (1991) and Redfearn *et al.* (1995) reported that during vegetative growth both ADIN and ADIN/TN were consistently higher

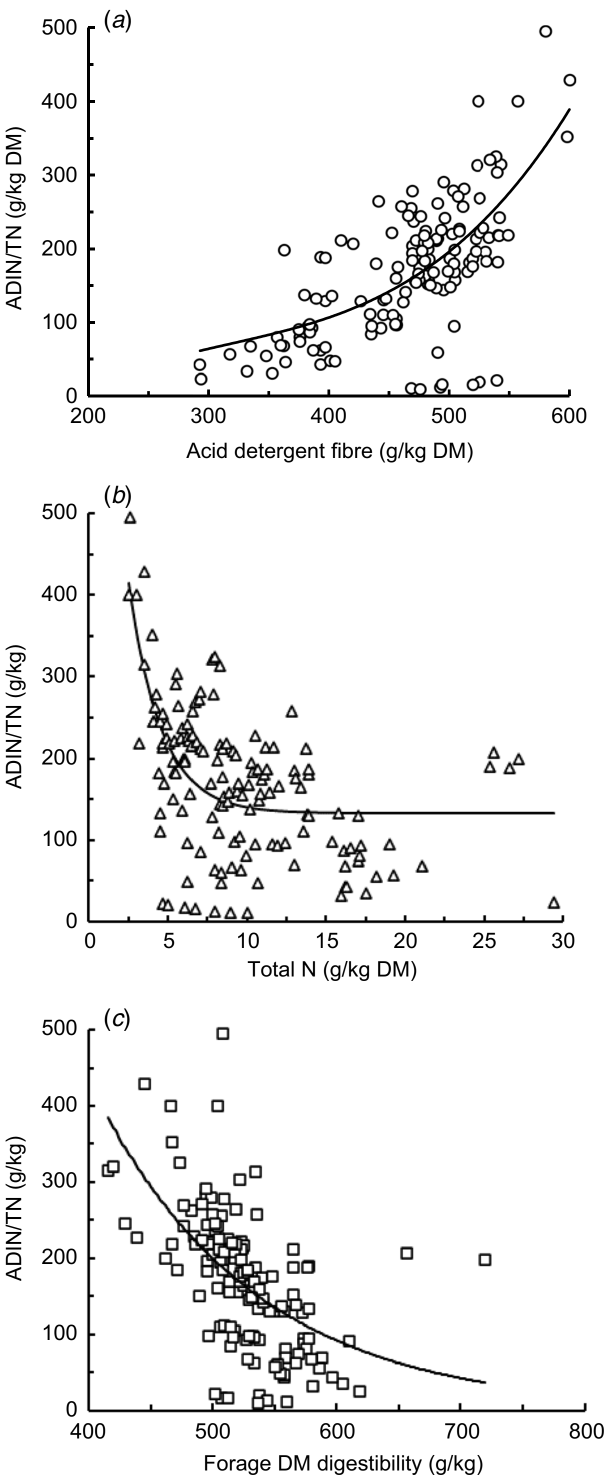

Fig. 2. Dataset 1: relationships in C_4 grasses between ADIN/TN ratio and (*a*) ADF concentration, (*b*) total N concentration, and (*c*) diet DM digestibility ($n = 143$). Equations are: (*a*) $Y = 31.5 + 3.23\exp(1.00786X)$ (RMSEP = 67.9, $R^2 = 0.43$, $P < 0.001$); (*b*) $Y = 132.5 + 952\exp(-0.4874X)$ (RMSEP = 74.0, $R^2 = 0.33$, $P < 0.001$); (*c*) $Y = -3.8 + 8836\exp(-0.00755X)$ (RMSEP = 74.3, $R^2 = 0.33$, $P < 0.001$).

in two C_4 grasses (*Andropogon gerardii* and *Panicum virgatum*) than in a C_3 grass (*Bromus inermis*), and the former study also found that these attributes could vary widely between years. However, contrasting results were reported by Mitchell *et al.* (1997), who studied two C_3 grasses and two C_4 grasses grown in the same environment during one summer. First, values of ADIN/kg DM and ADIN/TN were generally higher than reported in the two studies cited above, and also increased with plant maturity; at the greatest maturity examined, there was a range from 226 to 408 g ADIN/kg TN among these C_3 and C_4 grasses. Second, there were large differences among the grass species such that values of ADIN/kg DM and ADIN/TN ranked in the order: *A. gerardii* (C_4) > *P. virgatum* (C_4) ≈ *B. inermis* (C_3) > *Thinopyrum intermedium* (C_3). In this experiment the differences between the two grass species within each of the C_3 and C_4 classes could be as great as differences between C_4 and C_3 classes. There is evidence that higher ADIN concentration and ADIN/TN ratio occur with low soil fertility and/or unfavourable environmental conditions for grass growth; Johnson *et al.* (2001) reported that the ADIN/TN ratio was generally low (<120 g N/kg TN) in C_4 grasses (*C. dactylon*, *Cynodon nlemfuensis* and *Paspalum notatum*) grown in a favourable environment. Differences between C_3 and C_4 grasses and changes with increasing plant maturity may be associated with the different anatomical structures and greater proportions of parenchymal bundle sheaths and epidermis in both the leaf and stem of the C_4 grasses (Wilson 1994), and with the generally higher content of ADF in C_4 grasses (Laetsch 1974; Akin and Burdick 1975; Akin 1989). It is clearly important to understand the circumstances under which the ADIN/TN ratio is high and TN is low in C_4 grass forages, but further investigation is needed to understand the reasons for the variation in the ADIN/TN ratio.

Limitations of the present study for examining the relationships between ADIN/TN ratio and other forage attributes were that neither sample set was designed for that purpose, and that the reported measurements were limited. The forages in Dataset 1 were harvested from pasture swards that usually contained a mix of C_4 perennial grass species in various stages of regrowth, or were hays obtained from commercial farms. The growth and physiological maturity of the grasses sampled varied with the grass species and with interactions such as with soils and rainfall events to provide a broad array of forages as required for the development of the F.NIRS calibrations. Similarly, Dataset 2 collating results for 305 forages from 30 published papers did not provide a rigorous or balanced sample set. Nevertheless the present study showed that, on average, lower quality C_4 grass forages containing ~>400 g ADF/kg, ~<10 g TN/kg or ~<530 g digestible DM/kg had much higher ADIN/TN ratios than generally found in higher quality C_4 grass forages, C_3 grass forages or legumes. It was

Table 3. Datasets 1 and 2: the percentage distribution of categories of acid detergent fibre (ADF) content and total N (TN) concentration in C_4 grasses, and the category mean measured concentration of N insoluble in ADF solution (ADIN) and ADIN/TN ratio.

Category	Observations		Measurement	
	n	Percentage	ADIN (g/kg DM)	ADIN/TN (g/kg)
Dataset 1				
ADF (g/kg DM) (*n* = 143)				
<400	28	20	1.54 (1.34)	86 (47)
400–450	15	10	1.48 (1.28)	132 (61)
450–500	56	39	1.42 (0.64)	175 (67)
>500	44	31	1.42 (0.63)	230 (99)
TN (g N/kg DM) (*n* = 143)				
<5	22	15	1.00 (0.32)	257 (109)
5–10	67	47	1.25 (0.59)	173 (82)
10–15	32	23	1.83 (0.68)	154 (55)
15–20	16	11	1.28 (0.51)	76 (31)
>20	6	4	3.77 (2.12)	146 (79)
DMD (g/kg) (*n* = 139)				
<500	27	20	1.38 (0.51)	255 (72)
500–530	57	41	1.40 (0.55)	187 (81)
530–560	31	22	1.18 (0.80)	113 (71)
>560	24	17	1.95 (1.57)	110 (61)
Dataset 2				
ADF (g/kg DM) (*n* = 105)				
<400	51	48	1.53 (0.87)	123 (96)
400–450	28	27	1.46 (0.95)	170 (158)
450–500	17	16	1.77 (0.80)	174 (52)
>500	9	9	2.81 (2.21)	277 (223)
TN (g N/kg DM) (*n* = 188)				
<5	4	2	1.23 (0.62)	329 (205)
5–10	54	29	1.16 (1.09)	158 (148)
10–15	45	24	2.06 (1.12)	165 (100)
15–20	46	24	2.40 (1.18)	140 (69)
>20	39	21	2.36 (1.48)	101 (69)

Distributions are also given for categories of dry matter digestibility (DMD) measured in Dataset 1 using near-infrared spectroscopy of faeces. Standard deviations are in parentheses.

also clear that among the low-quality C_4 grasses there was great variability in the ADIN/TN ratio, ranging from 11 to 495 g ADIN/kg TN. Wilson (1982, 1994) has provided excellent qualitative descriptions of the structure and anatomy of C_4 grasses, C_3 grasses and dicotyledonous forages and how these are likely to be influenced by plant part, age of individual leaves and stem, water stress and temperature. However, it was not possible to relate such factors to the species, growing conditions or anatomical structure of the grasses in Datasets 1 and 2. High soil N availability during grass growth would be expected to increase the TN concentration and proportion of soluble N; however, as stated in the *Materials and methods*, the forages in Dataset 1 were generally not from fertilised soils. Also Dataset 2 did not include samples from treatments where the grass had been fertilised with N. It seems likely that better understanding of effects of forage species and environment will explain at least some of the variation in ADIN/TN ratio among C_4 grasses and provide the reasons why this ratio is high in highly fibrous C_4 grasses. This requires further investigation. Until further information is available it seems reasonable that for senesced C_4 grasses, defined, for example, as containing >400 g ADF/kg, <10 g TN/kg or <530 g digestible DM/kg, nutritional recommendations should assume that 20–30% of the TN is not available to the ruminant.

Importance of indigestible N in forages for ruminant production systems

The finding that a substantial proportion of the TN in mature C_4 grasses is indigestible will be most important in circumstances where diets are based on such forages that are marginal or deficient in N. One such situation is in low-input, low-output tropical production systems where ruminants are highly dependent on physiologically mature or senesced C_4 grass forages for many months of the annual cycle. This often occurs in rangeland production systems in the seasonally dry tropics of the northern Australian, African and South American rangelands where the only pastures available during the dry season are physiologically mature or senesced and weathered (Winks 1984; Hogan 1996). Many of these C_4 grass forages, such as those in the present Dataset 1 and in the database of Norton (1982), contain <10 g N/kg DM and have low DMD, so that the ADIN/TN ratio is expected to be high. In addition, Aumont *et al.* (1995) reported that in a large, normally distributed database of forages from the humid tropics in the Caribbean and La Reunion (*n* = 1313), the means and standard deviations indicated that ~30% of the forages contained >400 g ADF/kg DM or <11 g N/kg DM. High ADIN/TN ratios are also likely to occur in C_4 crop residues (e.g. maize, sorghum and millet stovers), which are important feedstuffs in many African crop–livestock systems (Duncan *et al.* 2013, 2016; Dejene *et al.* 2022). Senesced tropical pastures or crop residues are usually deficient in TN for ruminants (Winks *et al.* 1979; Hennessy and Williamson 1990; Kennedy *et al.* 1992; Coates and Dixon 2008; Dixon and Coates 2010). Lower than expected availability of the TN in such forages will require reconsideration of the need for, and responses of ruminants to, supplementary rumen-degradable N. In low-input systems such as those described above, the provision of N supplements usually incurs substantial increases in input costs and necessary management skills, and especially where urea non-protein

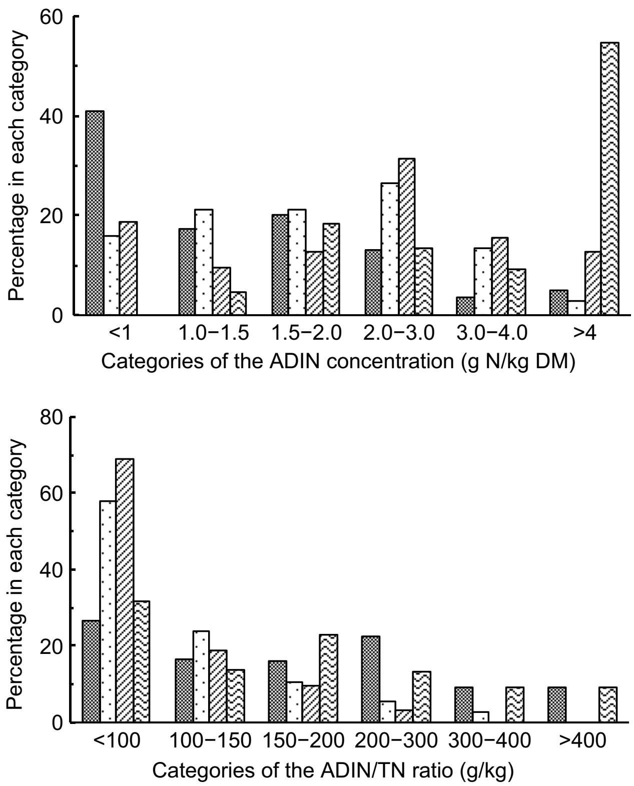

Fig. 3. Dataset 2: distribution of samples of forage classes (left to right): C_4 grasses (n = 187), C_3 grasses (n = 45), herbaceous tropical legumes (n = 51), and temperate legumes (n = 22) across categories of ADIN concentration, and categories of ADIN/TN ratio. Sources of data were: Blasi *et al.* (1991); Bowen (2004); Brown and Pitman (1991), Buckner *et al.* (2013), Chaurasia *et al.* (2006), Coblentz *et al.* (2004), da Silva *et al.* (2013), Das *et al.* (2015), Erasmus *et al.* (1990), Fondevila *et al.* (2002), Foster *et al.* (2012), Gemeda and Hassen (2014), Gupta *et al.* (2011), Hannah *et al.* (1991), Johnson *et al.* (2001), Juarez Lagunes *et al.* (1999), Kabuga and Darko (1993), McLennan (1997), Mitchell *et al.* (1997), Mupangwa *et al.* (2003), Negi *et al.* (1988*a*, 1988*b*), Nogueira Filho *et al.* (2000), Ogden *et al.* (2006), Peripoli *et al.* (2016), Prakash *et al.* (2003), Salazar-Cubillas and Dickhoefer (2021), Scarbrough *et al.* (2002), Singh *et al.* (2002, 2012), Wang *et al.* (2015), Zhao and Cao (2004).

N is used to provide supplementary N. The development and availability of F.NIRS in ruminants to measure diet attributes (Stuth *et al.* 1999; Coates 2004; Dixon and Coates 2009, 2015) provides opportunity for commercial farms to routinely monitor the ADF, TN, DMD and metabolisable energy of the diet of grazing cattle, and to use these measurements to estimate the proportion of indigestible N.

Conclusions

The study established that in highly fibrous (>400 g ADF/kg DM) and low TN (<10 g TN/kg DM) C_4 grass forages, the ADIN/TN ratio was highly variable, ranging from negligible to ~50%, but averaging ~20%. This greater than expected proportion of indigestible forage TN bound to plant cell walls requires consideration when evaluating the adequacy of TN in the diets of ruminants depending on such forages. Further investigation is needed to identify the grass species, environmental conditions and circumstances where the proportion of indigestible ADIN in TN in such forages is of practical nutritional importance.

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Data availability. The data that support this study may be shared upon reasonable request to the corresponding author if appropriate.

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