

Forage production and potential nutritive value of 24 shrubby *Indigofera* accessions under field conditions in South Africa

ABUBEKER HASSEN¹, N.F.G. RETHMAN²,
Z. APOSTOLIDES³ AND W.A. VAN NIEKERK¹

¹ Department of Animal and Wildlife Sciences,

² Department of Plant Production and Soil Science, and

³ Department of Biochemistry, University of Pretoria, Pretoria, South Africa

Abstract

Twenty-four shrubby *Indigofera* accessions from 7 species were evaluated in terms of their forage production, potential nutritive value and indospicine levels in the forage biomass over two growing seasons. Eighteen seedlings per plot were transplanted into field plots measuring 1.5 × 3 m in January 2003 at spacings of 50 cm between and within rows, with 3 replicates. In both seasons, differences between and within species for plant height, canopy spread diameter, fodder yield and leaf percentage of the biomass were significant ($P < 0.05$). *I. amorphoides* 7570, *I. cryptantha* 7070 and *I. arrecta* 7709 were superior in terms of forage yield in the first season, while *I. amorphoides* 7549, *I. cryptantha* 7067 and *I. arrecta* 10350 were superior in the subsequent season. Yields as high as 21 t/ha DM (total) and 5 t/ha DM (leaf) were obtained in some accessions in Year 2. Crude protein concentrations were high for *I. cryptantha* (29.8%) and *I. amorphoides* (27.7%), while the lowest were recorded for *I. coerulea* (15.9%) and *I. vicioides* (20.1%). Phosphorus concentrations in the forage biomass were higher for *I. cryptantha* (0.37%), *I. brevicalyx* (0.35%) and *I. amorphoides* (0.33%) than for *I. costata* (0.23%). The *in vitro* organic matter digestibility ranged from 74.8% for *I. amorphoides* to 63.8% for *I. brevicalyx*.

Indospicine levels in the forage biomass varied dramatically both between and within species. Concentrations were insignificant (0–2 mg/kg DM) in *I. brevicalyx*, and reached 706 mg/kg DM in *I. vicioides*. Within *I. arrecta*, levels varied 10-fold (26–289 mg/kg DM). The variation, which exists in this genus in the various parameters, indicates considerable opportunity to select accessions for possible feeding studies with animals to determine acceptability and possible deleterious effects.

Introduction

Generally, indigenous fodder trees and shrubs are valuable sources of feed during the driest months and drought periods across the semi-arid and arid areas of the tropics and subtropics. However, many are not useful as feed supplements as they contain anti-nutritional compounds, which are toxic to rumen microbes or to the animal, or their metabolic products are toxic (D'Mello 1992; Lowry *et al.* 1996).

Indigofera spp. display excellent adaptation to a range of environments (A. Hassen *et al.*, unpublished data), and possess diverse morphological and agronomic attributes, significant to their use as forage and cover crops (Hassen *et al.* 2006). Shrubby types generally produce more biomass than prostrate types and there is remarkable variation between and within species (Hassen *et al.* 2006). However, little is known about their variation in terms of winter survival, forage production in subsequent seasons, potential nutritive value and anti-nutritional compounds, which may limit the feeding value of the forage. Previous studies have indicated that some species, *e.g.* *Indigofera spicata*, contain the free amino acid, indospicine, which causes hepato-toxicity when this species is grazed by cattle (Norfeldt *et al.* 1952) or fed to chicks (Britten *et al.* 1963), rabbits (Hutton *et al.* 1958a), mice (Hutton *et al.* 1958b) or rats (Christie *et al.* 1975). Among collections

evaluated in Australia, genetic variation between and within species was significant (Williams 1981; Strickland *et al.* 1987), suggesting the need for screening more genetic material before promoting the species widely as a forage crop.

Chemical analyses, particularly in combination with *in vitro* digestibility and the determination of the indospicine levels in the leaf biomass, can help to assess the potential nutritive value of species/accessions at a preliminary stage of evaluation for their use as forage plants. The present study evaluated 24 shrubby accessions of *Indigofera*, from 7 species, to assess variation in forage biomass production and winter survival at Pretoria, as well as potential nutritive value of the leaf biomass as a forage source for both livestock and game.

Materials and methods

Location, field layout and management

The field experiment was carried out on the Hatfield Experimental Farm, University of Pretoria (28.11°E, 25.44°S; 1370 m asl), South Africa. The soil at the experimental site is classified as a sandy-loam, with a pH of 4.2, P concentration of 29 mg/kg, K of 73 mg/kg, Ca of 158 mg/kg, Mg of 38 mg/kg and Na of 11 mg/kg. Seeds of 24 shrubby *Indigofera* accessions were sown in trays in a nursery. After establishment, 54 seedlings of each accession were transplanted into field plots in January 2003. Eighteen seedlings were planted per 1.5 m × 3 m plot with a spacing of 50 cm between rows and plants within rows. There were 3 replications. Spacings of 50 cm and 100 cm were maintained between adjacent plots and blocks, respectively. The plants were irrigated twice per week for 2 hours depending on rainfall events. Plots were kept weed-free by hand-pulling.

A total of 5 middle plants, including 1 border plant, per plot were used to estimate dry matter yield with a harvestable plot area of 1.25 m². In the first growing season (2002–2003), all accessions were harvested at the 50% flowering stage to a height of 15 cm. The flowering time ranged between 104 and 165 days after planting. Subsequently, all plots were cut to the same height before the commencement of winter (June 2003), and left to grow to determine winter survival and biomass production in the subsequent season. In the 2003–2004 growing season, however,

all accessions were harvested at the same time (March 15–18, 2004). The harvested material was separated into leaf and stem components, which were dried in a forced-draught oven at 70°C for 48 hours to determine moisture concentration in the biomass.

Chemical composition and in vitro digestibility

Samples of leaf biomass harvested in 2003 were milled to pass through a 1 mm sieve and representative subsamples were stored in airtight containers for subsequent laboratory analyses. The DM, ash, P and N concentrations in the samples were determined following standard procedures (AOAC 2000). Crude protein (CP) was determined as N concentration × 6.25. *In vitro* organic matter digestibility (IVDOM) was determined by the Tilley and Terry (1963) procedure, as modified by Engels and van der Merwe (1967).

Indospicine determination

Indospicine analyses were performed on dried and milled leaf material in triplicate. The analysis involved 3 stages: plant extraction, solid phase extraction and ninhydrin test. The free amino acids were extracted as described by Pollitt *et al.* (1999). Since indospicine accumulates in the leaves, it comprises the major portion of the free amino acids. To improve method accuracy, the positively charged indospicine and other positive amino acids in minor amounts were purified with solid phase extraction as described in the Strata-X method Manual (Huq *et al.* 2003). Subsequently, the indospicine was determined with the ninhydrin method (Plumer 1978). The absorbance of 200 µL sample mixture was read in an ELISA plate using Multiskan Ascent V1.24 at 550 nm wavelength. A standard curve of the absorbance against arginine concentration was prepared, from which the unknown indospicine concentration was determined. This method provided a >70% recovery on 2 mg of arginine dissolved in the loading buffer (0.01 M carbonate buffer pH=10).

Statistical analyses

All studied parameters were subjected to analysis of variance to investigate the effects of replication, species and accession nested within a

species using Proc GLM of SAS (2001). Where the F ratio showed significance for either species or accession nested within a species, differences between least squares means were tested using the LSD test, which computes probabilities for all pair-wise differences.

Results

Biomass production and winter survival

There was wide variation ($P < 0.05$) both between and within *Indigofera* spp. in plant height, canopy spread diameter, total biomass yield and leaf biomass in both the first (2002–2003) and second (2003–2004) seasons (Tables 1 and 2). In the first season, average plant height for different species ranged from 17.3 cm (*I. brevicalyx*) to 91.9 cm (*I. arrecta*); canopy spread diameters from 19.7 cm (*I. costata*) to 78.4 cm (*I. arrecta*); total biomass yield from 97 kg/ha DM (*I. brevicalyx*) to 2728 kg/ha DM (*I. arrecta*); and potentially edible biomass (leaf biomass) from 74 kg/ha DM (*I. brevicalyx*) to 1150 kg/ha DM (*I. arrecta*). The percentage leaf in the total biomass was highest ($P < 0.05$) for *I. vicioides* (87.1%) and lowest for

I. arrecta (45.8%). Similar trends were observed in the second season, except that higher values were recorded for *I. arrecta* in terms of agronomic parameters (Table 2). Seedling survival 2 months after transplanting (Figure 1a) and winter survival (after 1 year) (Table 2) varied significantly ($P < 0.05$) between species. Generally, winter survival was highest for *I. brevicalyx*, *I. arrecta* and *I. cryptantha*, followed by *I. vicioides* and *I. amorphoides* (Table 2).

In 2002–2003, within the accessions of *I. amorphoides*, Accession 7557 was the tallest, but 7570 was superior in terms of leaf and total biomass yields (Table 3). Similarly, accessions of *I. arrecta* exhibited remarkable variation in terms of plant height, leaf yield and total biomass yield (Table 3). Seven accessions (7709, 7850, 7570, 10339, 10350, 8644 and 7598) gave total yields in excess of 3000 kg/ha and leaf yields in excess of 1000 kg/ha.

In the 2003–2004 season, intra-species variability between accessions of *I. amorphoides* was significant ($P < 0.05$). *I. amorphoides* 7549 and 7521 produced above 3500 kg/ha total DM and 1500 kg/ha leaf DM (Table 4). Accession 7557 produced very poorly. Within accessions of

Table 1. Inter-species variation in a collection of *Indigofera* species for plant height, mean canopy spread diameter, leaf percentage and % survival, leaf and total dry matter yields in the first season (2002–2003).

Species	Plant height (cm)	Canopy spread diameter (cm)	Leaf percentage	% survival after one year	Leaf dry matter yield (kg/ha)	Total dry matter yield (kg/ha)
<i>I. amorphoides</i>	65.9b (± 3.20) ¹	73.9a (± 5.78)	70c (± 2.2)	93b (± 3.2)	747b (± 112)	1165bc (± 190)
<i>I. arrecta</i>	91.9a (± 2.07)	78.4a (± 4.38)	46d (± 1.4)	97b (± 2.0)	1150a (± 72)	2728a (± 123)
<i>I. brevicalyx</i>	17.3d (± 5.07)	30.1bc (± 8.24)	79ab (± 3.5)	100a (± 5.0)	74d (± 177)	97d (± 301)
<i>I. coerulea</i>	26.3c (± 7.16)	20.8bc (± 20.6)	61cd (± 4.9)	39d (± 7.1)	127cd (± 251)	203d (± 425)
<i>I. costata</i>	19.2d (± 7.16)	19.7c (± 11.65)	64cd (± 4.9)	64cd (± 7.1)	196bcd (± 251)	314cd (± 425)
<i>I. cryptantha</i>	37.9c (± 5.69)	44.9b (± 11.87)	70bc (± 3.9)	99ab (± 5.6)	537bcd (± 199)	810ab (± 338)
<i>I. vicioides</i>	19d (± 8.84)	19.6bc (± 20.67)	87a (± 6.1)	82bc (± 8.7)	1120bc (± 309)	1411bc (± 524)

¹ Values within columns followed by different letters differ significantly ($P < 0.05$).

Table 2. Inter-species variation in a collection of *Indigofera* species for plant height, mean canopy spread diameter, leaf percentage and % survival, leaf and total dry matter yields in the second season (2003–2004).

Species	Plant height (cm)	Canopy spread diameter (cm)	Leaf percentage	% survival at end of year	Leaf dry matter yield (kg/ha)	Total dry matter yield (kg/ha)
<i>I. amorphoides</i>	59b (± 5.1) ¹	99.3c (± 6.83)	47.3b (± 1.80)	52b (± 4.3)	1255bc (± 295)	2481c (± 1209)
<i>I. arrecta</i>	201a (± 3.3)	127.3b (± 4.41)	20.5c (± 1.10)	97a (± 2.8)	3193a (± 180)	16620a (± 738)
<i>I. brevicalyx</i>	16c (± 8.1)	53.9e (± 10.8)	63a (± 2.57)	100a (± 6.9)	273c (± 420)	431c (± 1230)
<i>I. coerulea</i>	15c (± 11.4)	12.2f (± 15.28)	66.7a (± 3.64)	26c (± 9.7)	29c (± 597)	43c (± 2446)
<i>I. costata</i>	58b (± 11.4)	86.2d (± 15.28)	44b (± 3.64)	23c (± 9.7)	416c (± 597)	1013c (± 2446)
<i>I. cryptantha</i>	73b (± 9.1)	154.8a (± 12.13)	26.7c (± 3.69)	90a (± 7.7)	2559ab (± 605)	9740b (± 2479)
<i>I. vicioides</i>	23c (± 14.1)	19.4ef (± 18.85)	41.5b (± 4.49)	57b (± 12.0)	1420bc (± 737)	3981bc (± 3020)

¹ Values within columns followed by different letters differ ($P < 0.05$).

I. arrecta, 10 produced more than 15 000 kg/ha total yield and 10 produced more than 2500 kg/ha leaf.

Nutritive value

While there were significant differences between and within species for ash, CP, P, *in vitro* organic matter digestibility (IVOMD) and indospicine concentrations in the leaves (Tables 5 and 6), the magnitude of differences was much smaller than for agronomic parameters. Ash concentration was, in general, lower in *I. cryptantha* (9.0%), *I. brevicealyx* (10.2%) and *I. arrecta* (10.5%) than in *I. amorphoides* (12.6%), *I. coerulea* (12.9%) or *I. costata* (13.4%) (Table 5). The highest levels of CP were recorded in *I. cryptantha* (29.9%) and *I. amorphoides* (27.7%), and the lowest in *I. coerulea* (15.9%) and *I. vicioides* (20.1%). Phosphorus concentration ranged from 0.22% in *I. vicioides* to 0.37% in *I. cryptantha*. *In vitro* organic matter digestibility ranged from 74.8% in *I. amorphoides* to 63.8% in *I. brevicealyx*. Indospicine concentration in the leaves also varied between species, ranging from undetectable levels in *I. brevicealyx* (0–2 mg/kg DM) to 706 mg/kg DM in *I. vicioides*. The levels of indospicine

in *I. coerulea* and *I. cryptantha* were low (23–35.4 mg/kg DM).

While variations between accessions within *Indigofera* spp. did occur, only indospicine levels showed major differences (Table 6). Indospicine concentrations showed the greatest variation in *I. arrecta* with a range of 26 mg/kg (7850) to 289 mg/kg (7709) (Table 6). Overall, 8 accessions recorded indospicine concentrations below 50 mg/kg.

Discussion

A great deal of diversity in forage production potential was demonstrated both within and between the *Indigofera* species. Among the species included in this study, *I. arrecta*, *I. vicioides*, *I. amorphoides* and *I. cryptantha*, in decreasing order, demonstrated relatively high forage yield potential in the establishment season, whereas the forage yield potentials of *I. costata*, *I. coerulea* and *I. brevicealyx* were generally inferior.

There was significant variation both between and within species in terms of nutritive value of the forage. The leaves contained medium to high levels of CP (15.9–29.9%). NRC (1985; 1989) suggested that the diet for mature beef cattle

Table 3. Variation in a collection of *Indigofera* species in terms of mean plant height, canopy spread diameter, mean leaf and total dry matter yields and leaf percentage in the first season (2002–2003).

<i>Indigofera</i> accessions	Plant height (cm)	Canopy diameter (cm)	Leaf yield (kg/ha)	Total yield (kg/ha)	Leaf percentage
<i>I. amorphoides</i> 7069	64.7	47.3	878	1394	65.6
<i>I. amorphoides</i> 7521	50.6	54.1	398	554	74.8
<i>I. amorphoides</i> 7549	54.1	53.9	294	382	77.5
<i>I. amorphoides</i> 7557	109.5	138.0	604	916	68.9
<i>I. amorphoides</i> 7570	50.8	76.2	1558	2578	60.5
<i>I. arrecta</i> 7524	66.0	55.1	984	2122	45.7
<i>I. arrecta</i> 7592	74.0	68.1	961	2189	45.3
<i>I. arrecta</i> 7598	70.9	90.1	1201	2944	41.1
<i>I. arrecta</i> 7709	152.7	68.1	1770	5175	36.1
<i>I. arrecta</i> 7850	100.2	84.9	1680	3358	50.2
<i>I. arrecta</i> 8644	126.3	97.1	1347	3824	32.5
<i>I. arrecta</i> 9045	75.4	82.4	702	2019	35.3
<i>I. arrecta</i> 10339	92.0	75.6	1421	3211	43.8
<i>I. arrecta</i> 10350	136.2	119.1	1419	4044	35.5
<i>I. arrecta</i> 10355	38.8	74.3	678	1062	64.7
<i>I. arrecta</i> 10478	87.6	65.5	725	1197	61.5
<i>I. arrecta</i> 10479	83.2	60.8	915	1594	58.1
<i>I. brevicealyx</i> 7815	20.4	34.2	91	121	77.2
<i>I. brevicealyx</i> 7848	14.3	25.9	57	73	80.5
<i>I. coerulea</i> 9004	26.3	20.8	127	203	61.1
<i>I. costata</i> 8712	19.2	19.7	196	314	63.5
<i>I. cryptantha</i> 7067	19.5	28.1	278	461	70.3
<i>I. cryptantha</i> 7070	56.4	61.7	795	1160	69.2
<i>I. vicioides</i> 10486	19.0	19.6	1120	1412	87.1
LSD (P<0.05)	19.9	26.6	695	1178	13.7

Table 4. Variation in a collection of *Indigofera* species in terms of mean plant height, canopy spread diameter, mean leaf and total dry matter yields and leaf percentage in the second season (2003–2004).

<i>Indigofera</i> accessions	Plant height (cm)	Canopy diameter (cm)	Leaf yield (kg/ha)	Total yield (kg/ha)	Leaf percentage
<i>I. amorphoides</i> 7069	81.0	102.2	981	2390	41.5
<i>I. amorphoides</i> 7521	52.2	125.2	1565	3516	46.4
<i>I. amorphoides</i> 7549	80.7	107.0	2614	4894	46.3
<i>I. amorphoides</i> 7557	31.0	42.3	16	32	50.2
<i>I. amorphoides</i> 7570	50.9	119.8	1099	2280	52.1
<i>I. arrecta</i> 7524	137.4	131.9	3201	13589	23.5
<i>I. arrecta</i> 7592	186.1	130.7	2774	19313	15.5
<i>I. arrecta</i> 7598	159.2	142.4	3023	19322	17.0
<i>I. arrecta</i> 7709	235.6	87.8	2280	17359	13.3
<i>I. arrecta</i> 7850	227.8	149.3	5269	21623	23.4
<i>I. arrecta</i> 8644	227.8	117.2	2780	15783	17.7
<i>I. arrecta</i> 9045	242.2	135.9	3119	18787	16.0
<i>I. arrecta</i> 10339	210.0	136.2	2810	15578	18.7
<i>I. arrecta</i> 10350	233.4	145.0	4063	21217	19.0
<i>I. arrecta</i> 10355	59.2	133.1	1937	5528	36.6
<i>I. arrecta</i> 10478	251.6	119.0	3394	15319	23.0
<i>I. arrecta</i> 10479	242.8	99.4	3658	16030	22.7
<i>I. brevicalyx</i> 7815	15.9	55.5	260	399	64.8
<i>I. brevicalyx</i> 7848	16.0	52.2	287	464	61.1
<i>I. coerulea</i> 9004	15.0	12.2	29	43	66.7
<i>I. costata</i> 8712	58.0	86.2	416	1013	43.9
<i>I. cryptantha</i> 7067	67.1	188.7	3358	11915	30.9
<i>I. cryptantha</i> 7070	79.7	121.0	1760	7565	22.5
<i>I. vicioides</i> 10486	22.8	19.4	1420	3981	41.5
LSD (P<0.05)	31.6	42.4	1654	6781	10.1

Table 5. Inter-species variation (\pm s.e.) in a collection of *Indigofera* species in terms of nutritive value parameters and indospicine concentration in leaf dry matter.

Species	DM	Ash	CP (%)	P	IVOMD	Indospicine (mg/kg DM)
<i>I. amorphoides</i>	89.5a ¹ (± 0.99)	12.6a (± 0.35)	27.7a (± 0.80)	0.33a (± 0.021)	74.8a (± 1.17)	180.8b (± 23.9)
<i>I. arrecta</i>	88.9a (± 0.64)	10.5b (± 0.22)	24.3b (± 0.49)	0.28ab (± 0.013)	70.6b (± 0.74)	126.1bc (± 15.54)
<i>I. brevicalyx</i>	89.4a (± 1.42)	10.2b (± 0.50)	22.4b (± 1.14)	0.35a (± 0.294)	63.8c (± 1.67)	2.0c (± 48.85)
<i>I. coerulea</i>	90.4a (± 2.48)	12.9a (± 0.88)	15.9c (± 1.99)	0.24ab (± 0.051)	69.9bc (± 2.93)	23.0c (± 59.74)
<i>I. costata</i>	91.4a (± 2.01)	13.4a (± 0.71)	22.7b (± 1.61)	0.23b (± 0.042)	65.5c (± 2.37)	135.9bc (± 48.20)
<i>I. cryptantha</i>	91.0a (± 2.04)	9.0b (± 0.72)	29.9a (± 1.63)	0.37a (± 0.042)	73.6ab (± 2.40)	35.4c (± 49.07)
<i>I. vicioides</i>	90.9a (± 3.55)	11.1ab (± 1.26)	20.1bc (± 2.84)	0.22ab (± 0.074)	60.9c (± 4.18)	705.6a (± 85.49)

¹ Means within a column followed by different letters differ significantly (P<0.05).

should contain a minimum of 7.0% CP, while high producing dairy cows required 19.0% CP. Almost all *Indigofera* species tested could potentially provide sufficient nitrogen to supplement low quality roughages for beef animals, while most of the species, except *I. coerulea* 9004 and *I. arrecta* 9045, could supply the CP requirements of high producing dairy cows. This assumes that no anti-nutritive compounds are involved.

The CP levels of these *Indigofera* accessions were generally higher than reported levels for other browse species, such as *Flemingia macrophylla* (Dzowela *et al.* 1995), *Acacia nilotica*, *Albizia lebbek*, *Butea monosperma* (Ramana *et al.* 2000), *Vernonia amygdalina* (El Hassen

et al. 2000), *Cassia sturtii* (Van Niekerk *et al.* 2004; Wilcock *et al.* 2004; Ventura *et al.* 2004), *Rumex linaria*, *Acacia salicina* and *Adenocarpus foliosus* (Ventura *et al.* 2004), and were comparable with those for *Cajanus cajan*, *Acacia angustissima*, *Calliandra calothyrsus*, *Gliricidia sepium* and *Sesbania macrantha* (Dzowela *et al.* 1995), *Leucaena leucocephala*, *Pongamia pinnata* (Ramana *et al.* 2000), *Medicago sativa*, *Sesbania sesban* (El Hassen *et al.* 2000), *Atriplex nummularia* (van Niekerk *et al.* 2004), *Sutherlandia microphylla*, *Tripteris sinuatum* (Wilcock *et al.* 2004) and *Bituminaria bituminosa* (Ventura *et al.* 2004). However, the apparently high CP levels in the *Indigofera* species, compared with most

Table 6. Variation in a collection of *Indigofera* species in terms of mean ash, CP, P, IVDOM and indospicine concentrations in the leaves in the establishment season (2002–2003).

<i>Indigofera</i> accessions	Ash	CP (%)	P	IVDOM (%)	Indospicine (mg/kg DM)
<i>I. amorphoides</i> 7069	11.8	26.0	0.37	80.0	194
<i>I. amorphoides</i> 7521	13.2	27.7	0.27	80.1	314
<i>I. amorphoides</i> 7549	11.1	28.7	0.34	72.7	126
<i>I. amorphoides</i> 7557	13.3	29.4	0.39	69.7	146
<i>I. amorphoides</i> 7570	13.4	26.6	0.26	71.7	124
<i>I. arrecta</i> 7524	10.4	18.5	0.20	69.2	29
<i>I. arrecta</i> 7592	11.9	26.0	0.37	72.0	41
<i>I. arrecta</i> 7598	11.0	25.3	0.29	72.4	60
<i>I. arrecta</i> 7709	8.2	26.7	0.23	65.0	289
<i>I. arrecta</i> 7850	10.2	21.0	0.23	65.4	26
<i>I. arrecta</i> 8644	7.8	22.5	0.27	70.4	268
<i>I. arrecta</i> 9045	10.9	16.3	0.23	72.2	46
<i>I. arrecta</i> 10339	10.1	27.7	0.30	70.6	217
<i>I. arrecta</i> 10350	10.7	22.5	0.29	69.8	108
<i>I. arrecta</i> 10355	11.6	29.6	0.40	75.5	56
<i>I. arrecta</i> 10478	11.4	27.8	0.30	74.8	174
<i>I. arrecta</i> 10479	12.2	27.3	0.28	70.2	198
<i>I. brevicealyx</i> 7815	10.7	19.4	0.40	62.2	8.8
<i>I. brevicealyx</i> 7848	9.6	25.5	0.30	65.5	0
<i>I. coerulea</i> 9004	12.9	15.9	0.24	69.9	23
<i>I. costata</i> 8712	13.4	22.6	0.23	65.5	136
<i>I. cryptantha</i> 7067	9.0	30.1	0.42	76.6	6
<i>I. cryptantha</i> 7070	9.1	29.7	0.33	70.7	65
<i>I. vicioides</i> 10486	11.1	20.1	0.22	60.9	706
LSD (P<0.05)	0.20	0.44	0.011	6.6	13.4

browse species, may be misleading, as the high levels of indospicine in some of the *Indigofera* accessions, along with other plant nitrogenous secondary metabolites, could mean that animals would either not consume the material or not utilise it effectively.

Similarly, the P concentration in the forage biomass was higher than that reported for *Acacia* species (Abdulrazak *et al.* 2000), and higher than the lowest level (0.20% DM) recommended to meet growth requirements of cattle (ARC 1980). The *in vitro* OM digestibility of the poorer species was 65.0%, while it was as high as 80.0% for the best.

Differences in chemical composition have a strong bearing on the potential use of leguminous multi-purpose fodder trees in feeding systems (Dzowela *et al.* 1997), as they may affect palatability and intake by livestock both within and between species and provenances. In *Indigofera* species, secondary plant metabolites could influence palatability and intake, and the level of indospicine is a useful indicator of the potential toxicity of the feed under examination. We have demonstrated both inter- and intra-species variation in indospicine concentration in leaves within these *Indigofera* accessions (2–750 mg/kg DM). The concentrations of indospicine recorded for most accessions (*I. brevicealyx*,

I. coerulea, *I. cryptantha*, *I. arrecta*, *I. costata* and *I. amorphoides*) were lower than the levels reported for *I. volkensii* CPI No 33819 (2000 mg/kg DM) and 33 different *I. spicata* (500–12 000 mg/kg DM) accessions (Aylward *et al.* 1987). However, the threshold level, detrimental to animals, has not been precisely determined, though in *I. nigritana* (CPI No. 89268), concentrations as low as 100 mg/kg DM have resulted in incipient liver lesions (Aylward *et al.* 1987). The same authors reported variability in toxicity of accessions in a rat bioassay study. Of 46 accessions tested, 13 accessions, from 7 species, were considered to be non-toxic, while all accessions of *I. spicata* depressed liveweight gain and caused varying degrees of liver damage in rats (Aylward *et al.* 1987). Having a low concentration of indospicine may not ensure the suitability of the species as a forage. In Australia, *I. schimperi* displayed good chemical composition and no problems in feeding trials with rats, but was poorly accepted in grazing trials with cattle and supported poor weight gains (Clem and Hall 1994; T.J. Hall, personal communication).

The data presented on biomass yields, winter survival, CP, *in vitro* digestibility and indospicine levels have demonstrated that some of the

Indigofera species/accessions under evaluation, have moderate to high biomass yields, high crude protein concentrations, high digestibilities and low indospicine concentrations in the leaves. This makes them potential candidates for use as protein supplements. However, chemical composition alone has limited value in predicting the nutritive value of a new feed, which may contain materials toxic to the animal. The presence of indospicine in some of the species in relatively large quantities may be a major constraint to their efficient utilisation by the animal. Future research needs to address how this may be overcome, if *Indigofera* species are to be used widely as forage plants. On the other hand, the remarkable variability observed in this study, both between and within species, in terms of CP, IVOMD and indospicine concentration, suggests the possibility of directly selecting accessions with high forage potential and feeding value for subsequent evaluation with target animals. Feeding studies with animals are needed before any recommendations can be made about the potential of any of these accessions as livestock feeds.

Acknowledgements

The support given by technical assistants in the Departments of Biochemistry (Mrs R. du Toit and Mrs S. van Wyngaardt) and Animal and Wildlife Sciences (Mrs Ferreira) while undertaking the laboratory analyses on the leaf samples is gratefully acknowledged, as are the inputs of the crop section on the Hatfield Experimental Farm.

References

- ABDULRAZAK, S.A., FUJIHARA, T., ONDIEK, J.K. and ØRSKOV, E.R. (2000) Nutritive value evaluation of some *Acacia* tree leaves from Kenya. *Animal Feed Science and Technology*, **85**, 89–98.
- AOAC (2000) *Official Methods of Analysis*. 15th Edn. (Association of Official Analytical Chemists, Inc.: Washington, DC).
- AGRICULTURAL RESEARCH COUNCIL (ARC) (1980) *The Nutrient Requirements of Ruminant Livestock*. (Commonwealth Agricultural Bureau: Farnham Royal).
- AYLWARD, J.H., COURT, R.D., HAYDOCK, K.P., STRICKLAND, R.W. and HEGARTY, M.P. (1987) *Indigofera* species with agronomic potential in the tropics. Rat toxicity studies. *Australian Journal of Agricultural Research*, **38**, 177–186.
- BRITTEN, E.J., PALAFOX, A.L., FRODYMA, M.M. and LYND, F.T. (1963) Level of 3-nitropropanoic acid in relation to toxicity of *Indigofera spicata* in chicks. *Crop Science*, **3**, 415–416.
- CHRISTIE, G.S., WILSON, M. and HEGARTY, M.P. (1975) Effects on the liver in the rat of ingestion of *Indigofera spicata*, a legume containing an inhibitor of arginine metabolism. *The Journal of Pathology*, **117**, 195–205.
- CLEM, R.L. and HALL, T.J. (1994) Persistence and productivity of tropical pasture legumes on three cracking clay soils (Vertisols) in north-eastern Queensland. *Australian Journal of Experimental Agriculture*, **34**, 161–171.
- D'MELLO, J.P.F. (1992) Chemical constraints to the use of tropical legumes in animal nutrition. *Animal Feed Science and Technology*, **38**, 237–261.
- DZOWELA, B.H., HOVE, L., TOPPS, J.H. and MAFONGGOYA, P.L. (1995) Nutritional and anti-nutritional characters and rumen degradability of dry matter and nitrogen for some multipurpose tree species with potential for agroforestry in Zimbabwe. *Animal Feed Science and Technology*, **55**, 207–214.
- DZOWELA, B.H., HOVE, L., MAASDORP, B.V. and MAFONGGOYA, P.L. (1997) Recent work on the establishment, production and utilization of multipurpose trees as a feed resource in Zimbabwe. *Animal Feed Science and Technology*, **69**, 1–15.
- EI HASSEN, S.M., LAHLOU KASSI, A., NEWBOLD, C.J. and WALLACE, R.J. (2000) Chemical composition and degradation characteristics of foliage of some African multipurpose trees. *Animal Feed Science and Technology*, **86**, 27–37.
- ENGELS, E.A.N. and VAN DER MERWE, F.J. (1967) Application of an *in vitro* technique to South African forages with special reference to the effect of certain factors on the results. *South African Journal of Agricultural Science*, **10**, 983–995.
- HASSEN, A., RETHMAN N.F.G. and APOSTOLIDES, Z. (2006) Morphological and agronomic characterisation of *Indigofera* species using multivariate analysis. *Tropical Grasslands*, **40**, 45–59.
- Huq, S., Dixon, A. and Teuscher, J. (2003) Simplified method development for the extraction of acidic, basic and neutral drugs with a single solid phase extraction sorbent- Strata™ X. *Application Note TN-004*. (Phenomenex Inc.: Torrance, CA USA) (http://www.sampleprep.com/bin/strata-X_product_information.pdf)
- HUTTON, E.M., WINDRUM, G.M. and KRATZING, C.C. (1958a) Studies on the toxicity of *Indigofera endecaphylla*. I. Toxicity for rabbits. *Journal of Nutrition*, **64**, 321–328.
- HUTTON, E.M., WINDRUM, G.M. and KRATZING, C.C. (1958b) Studies on the toxicity of *Indigofera endecaphylla*. II. Toxicity for mice. *Journal of Nutrition*, **65**, 429–440.
- LOWRY, J.B., MCSWEENEY, C.S. and PALMER, B. (1996) Changing perceptions of the effect of plant phenolics on nutrient supply in the ruminant. *Australian Journal of Agricultural Research*, **47**, 829–842.
- NATIONAL RESEARCH COUNCIL (1985) *Nutrient Requirements of Beef Cattle*. 6th Revised Edn. (National Academy of Science: Washington, DC).
- NATIONAL RESEARCH COUNCIL (1989) *Nutrient Requirements of Dairy Cattle*. 6th Revised Edn. (National Academy of Science: Washington, DC).
- NORFELDT, S., HENKE, L.A., MORITA, K., MATSUMOTO, H., TAKAHASHI, M., YOUNGE, O.R., WILLERS, E. and CROSS, R.F. (1952) Feeding tests with *Indigofera endecaphylla* Jacq (creeping indigo) and some observations on the poisonous effects on domestic animals. *University of Hawaii, Agricultural Experiment Station Technical Bulletin*, Number 15.
- PLUMER, D.T. (1978) *An Introduction to Practical Biochemistry*. 2nd Edn. (McGraw Hill: London, UK).
- POLLITT, S., HEGARTY, M.P. and PASS, M.A. (1999) Analysis of the amino acid indospicine in biological samples by high performance liquid chromatography. *Natural Toxins*, **7**, 233–240.
- RAMANA, D.B.V., SINGH, S., SOLANKI, K.R. and NEGI, A.S. (2000) Nutritive evaluation of some nitrogen and non-nitrogen fixing multipurpose tree species. *Animal Feed Science and Technology*, **88**, 103–111.
- SAS (2001) *SAS/STAT User's Guide*. Version 8.2. (Statistical Analysis Systems Institute Inc.: Cary, NC).
- STRICKLAND, R.W., LAMBOURNE, L.J. and RATCLIFF, D. (1987) A rat bioassay for screening tropical legume forages and seeds for palatability and toxicity. *Australian Journal of Experimental Agriculture*, **27**, 45–53.

- TILLEY, J.M.A. and TERRY, R.A. (1963) A two stage technique for *in vitro* digestion of forage crops. *Journal of the British Grassland Society*, **18**, 104–111.
- VAN NIEKERK, W.A., SPARKS, C.F., RETHMAN, N.F.G. and COERTZE, R.J. (2004) Qualitative characteristics of some *Atriplex* species and *Cassia sturtii* at two sites in South Africa. *South African Journal of Animal Science*, **34**, 108–110.
- VENTURA, M.R., CASTAÑÓN, J.I.R., PIELTAIN, M.C. and FLORES, M.P. (2004) Nutritive value of forage shrubs: *Bituminaria bituminosa*, *Rumex lunaria*, *Acacia salicina*, *Cassia sturtii* and *Adenocarpus foliosus*. *Small Ruminant Research*, **52**, 13–18.
- WILCOCK, T.E., VAN NIEKERK, W.A., RETHMAN, N.F.G. and COERTZE, R.J. (2004) A comparison of *Cassia sturtii*, *Tripteris sinuatum* and *Sutherlandia microphylla*: three fodder shrubs applicable to revegetation of degraded rangeland in the Northern Cape province. *South African Journal of Animal Science*, **34**, 114–116.
- WILLIAMS, M.C. (1981) Nitro compounds in *Indigofera* species. *Agronomy Journal*, **73**, 434–436.

(Received for publication August 2, 2006; accepted October 1, 2007)

NB Editor's note: *Indigofera* spp. are considered undesirable plants in Australia. Toxic native *Indigofera* spp. cause problems with grazing livestock and the introduced African species *I. schimperi* is a potential serious weed. It was included in State-wide evaluation trials as laboratory analyses indicated good chemical composition and feeding trials with rats produced no liver lesions. In subsequent

field trials, it displayed poor palatability and produced poor animal performance. Since it grows from both root suckers and seed and thrives on good clay soils, especially when its deep rooting allows it to thrive when other plants are dying, it poses a serious weed threat. QDPI&F and CSIRO are currently conducting a 6-year study to eradicate this species from old evaluation sites.