EFFECTS OF NITROGEN FERTILIZATION AND HARVEST DATE ON FORAGE QUALITY FACTORS AND PROTEIN FRACTIONS

C.R. Johnson*, B.A. Reiling*, P. Mislevy†, M.B. Hall††

*Department of Animal Sciences, ††Department of Dairy and Poultry Sciences, University of Florida, Gainesville 32611-0910 and †Department of Agronomy, Range Cattle Research and Education Center, Ona 33865-9706

INTRODUCTION

Cow-calf operations are the primary focus of beef production in Florida, primarily due to the year-round availability of forages. Tropical forages (C_4) commonly used in beef production include bahiagrass, bermudagrass, and stargrass, and these forages are metabolically efficient. However, the efficient plant metabolism and utilization of nutrients results in a lower percentage of those nutrients being available to the animal (Brown, 1978), when compared to temperate forages. Tropical grasses exhibit peak yields during mid-summer (Sollenberger et al., 1989), however a depression in animal performance is realized during the same time, primarily due to forage quality being limiting (Sollenberger et al, 1989; Sollenberger and Jones, 1989; Rusland et al., 1988). Application of nitrogen fertilization on warm-season forages increases forage dry matter yield (Prine and Burton, 1956; Caraballo et al, 1997) and the quantity of forage crude protein (Prine and Burton, 1956; Harvey et al., 1996).

A challenge to researchers has been to accurately predict forage nutrient supply to the animal, particularly with warm-season forages. The NRC's *Nutrient Requirements of Beef Cattle* has attempted (NRC, 1996) to more accurately characterize protein and carbohydrate fractions within ruminant feeds, with incorporation of the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992). Previous research characterized the protein fractions of various species of forages (Krishnamoorthy et al., 1982; Brown and Pitman, 1991; Elizalde et al., 1999), however, information on the effect of fertilization and harvest date on the protein fractions of tropical forages is limiting. The current study was designed to evaluate the effects of managerial practices (nitrogen fertilization, harvest date) on dry matter yield, digestibility, and protein fractions of tropical forages commonly used in Florida beef production.

MATERIALS AND METHODS

This experiment was conducted in conjunction with a tropical grass x nitrogen (N) fertilization experiment at the Range Cattle Research and Education Center in Ona, FL (27° 25'N, 81° 55' W) during 1997 and 1998. The experiment was arranged as a split-split plot with three tropical grass species as the main plot, arranged in a randomized block design with four field replications. The

Presented at the 11th Florida Ruminant Nutrition Symposium, Gainesville, January 13-14, 2000

tropical grass cultivars studied were *Cynodon dactylon* (L) (Tifton 85 bermudagrass), *Cynodon nlemfuensis* Vanderyst var. *nlemfuensis* (Florona stargrass), and *Paspalum notatum* (Pensacola bahiagrass). Split-plot treatments included five N (ammonium nitrate) rates (0, 39, 78, 118 and 157 kg/ha) applied directly after each harvest.

Plots were established in 1996. Bermudagrass and stargrass were vegetatively planted using sprigs (3360 kg/ha) on August 29, 1996. Bahiagrass was planted on September 4, 1996 at a seeding rate of 45 kg/ha. Per hectare, all treatments received 24.5 kg P, 93.0 kg K, 2.8 kg Zn, 2.8 kg Fe, 2.8 kg Cu, 2.8 kg Mg, and .28 kg B on March 21, 1997. In 1998, the same quantity of fertilizer was applied in split applications, the first on May 5, 1998 and the second on July 28, 1998. Plots (n = 60) measured 6.1 m × 7.6 m with 3 m alleyways between each main plot cultivar. Winter growth was removed from all plots in early May and harvested at 28 d intervals beginning early June and continuing through September.

Forages were harvested using a rotary blade plot harvester that cut a .49 m × 3.08 m swath leaving a stubble height of 7.6 cm. Fresh forage weights were recorded for yield determinations, and a forage sub-sample was taken for subsequent laboratory analyses. Samples were dried in a forced air oven at 55°C. After drying and DM determination, all samples were ground to pass through a 1 mm screen using a Christy-Norris mill (Christy and Norris Limited, Chelmsford, England) for the 1997 harvests and a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) for the 1998 harvests. Ground samples were stored in sealed plastic bags.

Response variables measured included total N, five protein fractions, NDF, ADF, in-vitro organic matter digestibility, and forage yield. Laboratory DM and ash determinations were conducted according to AOAC, Official Methods of Analysis (1960), using .5 g of sample. Protein fractions determined were non-protein nitrogen (Fraction A); true protein rapidly degraded in the rumen (Fraction B_1); true protein moderately degraded in the rumen (Fraction B_2); true protein associated with the cell wall and slowly degraded in the rumen (Fraction B_3); and insoluble protein (Fraction C; Chalupa et al., 1991). Protein fractions were determined using the procedures of Licitra et al. (1996), with the following modifications. For the non-protein nitrogen procedure, the trichloracetic acid method was used with .75 g of sample and 75 ml of deionized water and 15 ml of 10% TCA. In the borate-phosphate buffer soluble protein method, .75 g of sample was used with 75 ml buffer and 1 ml of sodium azide solution. Sample sizes for these two methods had to be increased to ensure that adequate amounts of nitrogen were present in the residue for accurate nitrogen determination. All nitrogen analyses were conducted using the micro-kjeldahl technique of Gallaher et al. (1975). Neutral detergent fiber and neutral detergent-insoluble nitrogen was determined using the method described by Van Soest et al. (1991) using Termamyl 120L (Novo Nordisk Biochem N.A. Inc., Franklinton, NC) as the heat-stable α-amylase. Acid detergent fiber and insoluble nitrogen was determined using the method described by Van Soest

(1973). In-vitro organic matter digestibility was determined using the two-stage technique of Tilley and Terry (1963).

Effects of fertilization, specie, and harvest date on yield, total N, IVOMD, ADF, NDF, and protein fractions were evaluated with analyses of variance for a randomized block design with a split-split plot arrangement of treatments (Snedecor and Cochran, 1967). Repeated measures analysis using the MIXED procedure of SAS (Littell et al., 1996; SAS, 1996) was conducted. Fertilization rate, forage specie and harvest date were fixed variables, field replications were determined the random variable, therefore each main effect (specie, fertilization, harvest date, and year) and subsequent interactions were nested within field replication. Polynomial contrasts were conducted to evaluate linearity of effects of fertilization, specie and harvest date.

RESULTS AND DISCUSSION

Forage dry matter yield

It is widely recognized that forage DM yield may be influenced by both managerial and climatic factors. Within each year of this study, variation in DM yield were observed for the different forage species, N fertilization rates, and harvest dates evaluated. Average forage DM yield for 1997 was 37% ($P < .01$) more than that harvested in 1998 (Figure 1). This response tends to be normal with tropical grasses due to a denser turf during the second growing season (P. Mislevy, personal communication). Still, the pattern of forage growth as affected by fertilization was similar for both growing seasons.

For all species and harvest dates, a quadratic effect ($P < .01$) of fertilization was observed (Figure 1, Table 1). Forage DM yield across all species at 0 kg N/(ha·cutting) was 712 ± 53 kg/ha, whereas peak forage yield (1627 ± 53 kg/ha) occurred with the application of 79 kg N/(ha·cutting). Thereafter, yield was essentially non-responsive to greater levels of N fertilization. Only during the early seasonal harvests (June) did additional N fertilization (118 or 157 kg/(ha·cutting)) further improve forage DM yield (Figure 2).

A fertilization rate by species interaction ($P < .01$) affecting forage DM yield was detected. At 0 kg of N/(ha·cutting), DM yield of all forage species was similar ($P > .05$). Numerically, however, DM yield of bahiagrass was 113 and 58 kg/ha more than bermudagrass and stargrass, respectively (Table 2). Upon application of N, however, both bermudagrass (at 39 kg N/(ha·cutting)) and stargrass (at 79 kg N/(ha·cutting)) exhibited greater yields than bahiagrass. Application of N at rates greater than 79 kg/(ha·cutting) did not further improve yields, but rather numerically reduced forage yield, especially of bahiagrass, which suggests that peak production is reached at this level. Prine and Burton (1956) reported maximum forage DM yields associated with the application of 267 kg/ha of N for the entire growing season, during a dry year and with 534 kg/ha of N, during a wet year. The average of these two values is equivalent to applying 79 kg of N/ha, after every cutting (5 applications). Figure 2 illustrates the effects of fertilization rate and harvest date on the DM yield for these three forages.

Harvest date exhibited a quadratic response on forage DM yield ($P <. 01$) (Table 1). Peak forage yield occurred during late June and July (Table 1), which corresponds to the growth patterns of warm-season grasses. Sumner et al. (1991) compared year-round bahiagrass yield on nine south Florida ranches, and reported that peak yields occurred during mid-summer. Additionally, Chambliss et al. (1999) and Mislevy (1999) reported peak yields for bermudagrass and stargrass, respectively, during mid-summer.

In-vitro organic matter digestibility

Increased levels of nitrogen fertilization exhibited a linear effect ($P < .01$) on the IVOMD of stargrass where nitrogen applications of 79 and 157 kg/(ha·cutting) increased ($P < .01$) the IVOMD of stargrass 5.6 and 11.3%, respectively, compared to 0 kg N/(ha·cutting) (Figure 3, Table 2). Bermudagrass, however, exhibited a quadratic effect ($P < .01$) of nitrogen fertilization on IVOMD. The IVOMD of unfertilized (N) bermudagrass averaged 58.0%, but a depression ($P < .05$) in IVOMD was associated with the application of 39 and 79 kg/(ha·cutting) of nitrogen. Upon application of 157 kg/(ha·cutting)of nitrogen, IVOMD rebounded to 60.0% ($P < .05$). In contrast, nitrogen fertilization had no effect on the IVOMD of bahiagrass. Nichols et al. (1990) found that application of 135 kg/ha of N to subirrigated meadow vegetation, decreased in-vitro dry matter digestibility (IVDMD) 5.2%, compared to a control of 0 kg of N/ha. However, McCormick (1974), reported no effect of N fertilization on the IVDMD of coastal bermudagrass, although a numerical decrease was observed. Harvey et al. (1996) also found no response to nitrogen fertilization on the IVDMD of bermudagrass pastures annually fertilized with either 456 or 873 kg/ha of nitrogen.

Harvest date revealed a quadratic response ($P < .01$) of IVOMD (Table 2). All species exhibited their greatest IVOMD during June (Harvest 1), however a mid-summer "slump" was noticed in the growth pattern of these forages. This occurred during July (Harvest 3) when the IVOMD of bahia-, bermuda-, and stargrass was reduced ($P < .05$) 9.9, 11.0, and 9.7%, respectively, compared to the IVOMD of Harvest 1 (early June). In Harvest 4 (August), IVOMD of the forages improved to 51.2, 57.3, and 54.8% for bahia-, bermuda-, and stargrass, respectively. Rusland et al. (1988) found a similar digestibility pattern in limpograss (*Hemarthria altissma*). Sollenberger et al. (1989) reported a depression in IVOMD of bahiagrass during the summer, with the greatest IVOMD values occurred in either the spring or fall. Temperature is negatively related with IVOMD in forages. A decrease in digestibility of 7.6% for bermudagrass and 12.9%, for bahiagrass, has been reported when temperatures increase from 26 to 35°C (Henderson and Robinson, 1982b). The negative relationship between digestibility and temperature may be attributed to a reduction in the leaf:stem ratio, associated with increased temperatures (Nelson and Volenec, 1995).

Acid-detergent Fiber

Harvest date exhibited a quadratic effect ($P < .01$) on ADF (Table 3) in these forages. Peak levels of ADF coincided with peak summer growth patterns and the depression observed in IVOMD and total N. Increased fiber concentrations are positively correlated with higher temperatures (Henderson and Robinson, 1982a and 1982b). Averaged across all harvest dates and fertilization treatments, bahiagrass possessed 6.4 and 8.3% more ($P < .05$) ADF than bermudagrass and stargrass, respectively. This difference was most pronounced during July and August, when the ADF of bahiagrass was 11.1 and 9.9% greater ($P < .01$) than stargrass. Brown and Pitman (1991) also reported higher concentrations of ADF in bahiagrass compared to limpograss (39.3 vs. 35.2%).

Fertilization varied in its effects on ADF levels of the different forage species. Percentage of ADF in bahiagrass was not influenced ($P > .05$) by fertilization. In contrast, the two *Cynodon* grasses had quadratic responses ($P < .01$, bermudagrass; $P < .05$, stargrass) to fertilization (Table 3). The ADF of both forages increased as N fertilization rates increased to 79 kg/(ha·cutting), after which fertilization appeared to decrease ADF. Previous research indicated that application of N has minimal effects on ADF concentrations of either warm-season or native range grasses (Cuomo and Anderson, 1996; Rogers et al., 1996).

Neutral-detergent Fiber

Nitrogen fertilization linearly decreased ($P < .01$) the NDF concentration (Table 4) of all three forage species. Stargrass exhibited a 6.4% reduction in NDF from 76.9 (0 kg N/(ha·cutting)) to 72.0% (157 kg N/(ha·cutting)), whereas bahiagrass and bermudagrass exhibited more modest reductions of approximately 2.7%. Previous research showed that increased N fertilization had little to no effect on NDF in bermudagrass or native grasses (Cuomo and Anderson, 1996; Rogers et al., 1996).

The effects of harvest date produced varying results, dependent on forage species. A linear increase ($P < .01$) of 2.8% in NDF was observed for bahiagrass, across the harvest season. In contrast, harvest date had a quadratic effect ($P < .05$) on bermudagrass, where peak levels of NDF occurred after Harvest 2 (late June). A cubic effect ($P < .01$) was observed for NDF in stargrass, with peak levels of 74.4 and 74.5% being observed in late June and September (Harvests 2 and 5, Table 4). Henderson and Robinson (1982a) reported a positive relationship between NDF and temperature for bermudagrass, which agrees with these data. In direct contrast with this study's data, however, Henderson and Robinson (1982a) reported a negative relationship between NDF and temperature in bahiagrass as NDF levels decreased 4 percentage units as temperature increased from 26°C to 35°C.

Total Nitrogen

Within each fertilization treatment and across all harvest dates, stargrass produced ($P < .01$) more total N (2.4%), expressed as a percentage of forage DM than either bermudagrass (2.2%) or bahiagrass (2.0%) (Table 5). However, within each fertilization treatment, a quadratic response (*P* < .01) was observed for harvest date on total N (Figure 4). A summer depression in total N content (*P* < .01) was associated with the late June and July harvest dates. Bahiagrass exhibited a loss of 7.1% total N as compared to the two *Cynodon* species, in which losses of 15.4% (bermudagrass) and 16.2% (stargrass) were detected during July, as compared to the early June harvest. By September, however, forage total N had regained 108.1 (1.98 vs. 2.14%), 102.1 (2.34 vs 2.39%), and 98.8% (2.60 vs. 2.57%) for bahia-, bermuda-, and stargrass (early June vs. September), respectively. Sumner et al. (1991) reported a 13.8% summer depression in bahiagrass regrowth crude protein concentration in July compared to a June harvest. Additionally, Rusland et al. (1988) reported a 25% loss in limpograss crude protein from early June to late July.

Bahia-, bermuda-, and stargrass total N concentration increased (*P* < .01) 69.4, 84.7, and 91.4%, respectively, when rate of nitrogen fertilization increased from 0 to 157 kg/(ha·cutting). Prine and Burton (1956) reported increases in bermudagrass crude protein of 21.0, 64.6, 83.6, and 111.5% as annual nitrogen fertilization rates increased from 0 to 89, 267, 534 and 882 kg/ha, respectively. Messman et al. (1992) reported a 32.0% increase in total N of bromegrass with a single application of 89 kg/ha of nitrogen.

Protein Fraction A: NPN

For each of the three forage species, not only did total N of the forage increase with fertilization, but the quantity and percentage of Fraction A (Table 6) relative to total N also increased linearly (*P* < .01). Nitrogen applications of 39, 79, 118, and 157 kg N/(ha·cutting) increased (*P* < .01) the quantity of Fraction A within bahiagrass by 19.4, 64.5, 80.6 and 125.8%, respectively. With no fertilization, NPN components comprised 21.2, 31.0, and 27.9% of the total N in bahia-, bermuda-, and stargrass, respectively. Upon application of 157 kg N/(ha·cutting), however, Fraction A represented 28.5, 40.0 and 42.1% of the total N in those same three forages. Rogers et al. (1996) reported increased levels of NPN (30.5 vs. 34.4% of total N) in bermudagrass pastures fertilized with increasing quantities of nitrogen (448 vs. 896 kg of N/(ha·yr)) from swine lagoon affluent. It has been reported (Hojjati et al., 1972; Bergareche and Simon, 1989; Follett and Wilkinson, 1995) that increased levels of nitrogen fertilization increase nitrate accumulation in the plant, however bermudagrasses are classified as low accumulators of nitrate.

Date of harvest exerted a quadratic effect (*P* < .01) on Fraction A for all forages. A depression of 11.8% of Fraction A was observed during July and August (Harvests 3 and 4), compared to early June (Harvest 1), across all species and fertilization rates (Table 7). Rogers et al. (1996) reported that NPN in bermudagrass was reduced 16.0% in August compared to the June harvest.

Protein Fraction B_1, B_2, B_3: True Protein

Greater rates of nitrogen application resulted in linear increases (*P* < .01) of 69.2, 84.6, and 72.2% in Fraction B_1 of bahia-, bermuda-, and stargrass, respectively (157 kg N/(ha·cutting) compared to no N fertilization). Additionally, increased nitrogen fertilization produced linear ($P < .01$) increases in Fraction B_2. A species x fertilization interaction was detected, with the two *Cynodon* grasses having at least 57.1% more (.33 vs. .21, % of forage DM; $P < .05$) Fraction B_2 protein than bahiagrass (Table 6). Cuomo and Anderson (1996) reported that ruminally degraded protein increased 12.1%, due to the application of 66 kg N/ha on native warm-season grasses. Rogers et al. (1996) observed an increase from 34.8 to 38.2% of soluble true protein, expressed as a percentage of total crude protein in bermudagrass, as a result of increasing annual fertilization from 448 to 896 kg N/ha.

For Fraction B_3, bahiagrass and bermudagrass had linear ($P < .01$) increases of 47.8 and 38.7%, respectively with the application of 157 kg N/(ha·cutting), whereas stargrass had a cubic ($P < .01$) response to increasing fertilization, most likely due to the slight depression seen in late June (Table 6). Additionally, Cuomo and Anderson (1996) reported that ruminally undegraded protein increased with the same increase in nitrogen fertilization.

Harvest date produced varying results, dependent upon forage specie, for each of the three true protein fractions. For Fraction B_1, harvest date had no effect on bahiagrass, yet produced a quadratic effect ($P < .05$) for bermudagrass and a linear decrease ($P < .05$) for stargrass (Table 7). Across all harvest dates, stargrass had a greater ($P < .01$) concentration (.24% of forage DM) of Fraction B_1 over bermudagrass and bahiagrass (both .18% of forage DM). Within Fraction B_2, harvest date produced a quadratic response ($P < .01$) in all species. Similar to total N concentration and IVOMD, a depression in Fraction B_2 was observed during Harvests 2 and 3 (late June and July). Rogers et al. (1996) reported a depression in soluble protein at the higher fertilization rate of 14.1% during August, for bermudagrass. In Fraction B_3, harvest date had no effect on bahiagrass, but produced a linear decrease ($P < .05$) in bermudagrass of 7.7% from early June to September. Stargrass had a quadratic response ($P < .05$), with a reduction in Fraction B_3 from late June to August of 27.4%, recovering 21.3% of the June harvest value, in September. A common trend observed for the two *Cynodon* grasses, included a reduction in Fraction B_3 associated with Harvests 3 and 4.

Protein Fraction C: Non-degradable Nitrogen

Fertilization had a quadratic effect ($P < .01$) on Fraction C of bahiagrass and stargrass, with peak levels occurring at higher N fertilization rates (Table 6). Fraction C in bermudagrass increased linearly ($P < .01$) 53.9% with the application of 157 kg N/(ha·cutting). Across all fertilization treatments, bahiagrass exhibited the greatest concentration (.25% of forage DM) of ADF-associated N. The two *Cynodon* grasses were similar, .17 and .18% of forage DM, for bermudagrass and stargrass, respectively. The higher level of Fraction C coincides with bahiagrass having a greater percentage of ADF as compared to bermudagrass and stargrass. Rogers et al. (1996) reported that fertilization had no effect on Fraction C of bermudagrass pastures, reporting means of 13.6 and 12.6% of total N as Fraction C, for nitrogen fertilization rates of 448 and 896 kg/(ha·yr), respectively. Zhang et al. (1995) observed a 55.3% decrease of ADF-N due to increasing nitrogen fertilization in annual ryegrass.

A quadratic ($P < .01$) response of Fraction C in all species, due to harvest date was observed, with peak levels occurring from late June through August. This coincided with peak levels of ADF, with which the protein in Fraction C is associated. Bahiagrass levels of Fraction C increased 55.0% from Harvest 1 to Harvest 3; the two *Cynodon* species were similar and experienced a change of less than half the magnitude of the bahiagrass (Table 7). Rogers et al. (1996) observed an increase of 33.3% in bermudagrass Fraction C, during August as compared to June.

IMPLICATIONS

Nitrogen fertilization is a key factor that affects forage yield and quality. Optimum yield was achieved for all forages at 79 kg N/(ha·cutting). Maximum total nitrogen was achieved at the higher fertilization treatments. The nitrogen pool available for rumen microbes to utilize in microbial protein synthesis is increased as fertilization rates increase. Energy supplementation could enhance microbial synthesis of protein from the larger pool of non-protein nitrogen, or escape protein could be supplemented to ensure an adequate supply of protein to the small intestine, when feeding heavily fertilized forages. The *Cynodon* grasses exhibited a larger percentage of Fraction A and less Fraction C, than bahiagrass, suggesting that these forages have a higher percentage of the forage nitrogen in a useable form for the animal. Depressions in digestibility, and digestible N fractions during summer months suggest that feed supplementation may be an appropriate strategy at that time.

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Table 1. Effect of forage specie, N fertilization and harvest date on forage dry matter yield[a,b]

Species	Harvest[d,e]	N fertilization rates[c], kg/(ha·cutting)					Mean	SEM[f]
		0	39	79	118	157	(harvest)	
Bahiagrass	1	527	1017	1048	1258	1119	994	133
	2	925	1301	1667	1687	1527	1121	133
	3	827	1956	1738	1560	1538	1524	133
	4	791	1378	1413	1261	1334	1235	133
	5	775	1380	1527	1529	1336	1309	133
Mean (fertilization)		769	1406	1479	1459	1371		
Bermudagrass	1	306	1024	1577	1847	2166	1384	133
	2	538	1895	2410	2473	2544	1972	133
	3	1010	2037	2076	2000	1802	1785	133
	4	767	1470	1568	1461	1132	1280	133
	5	659	1340	1477	1427	1395	1260	133
Mean (fertilization)		656	1553	1822	1842	1808		
Stargrass	1	309	833	1387	1666	1920	1223	133
	2	934	1523	2102	2349	2383	1858	133
	3	729	1526	1640	1605	1464	1393	141
	4	790	1330	1400	1524	1428	1294	133
	5	791	1342	1376	1423	1313	1249	133
Mean (fertilization)		711	1311	1581	1713	1702		

[a]LS means of forage dry matter yield, kg/ha.

[b]Probability for effects in the model: fertilization, $P < .01$; species, $P < .01$; harvest date, $P < .01$; fertilization*species, $P < .01$; fertilization*harvest date $P < .01$; species*harvest date, $P < .01$.

[c]Quadratic effect of fertilization, across all species and harvest dates, $P < .01$.

[d]Quadratic effect of harvest, across all species and fertilization rates, $P < .01$.

[e]Harvest 1, early June; harvest 2, late June; harvest 3, July; harvest 4, August; harvest 5, September.

[f]Greatest standard error of treatment means (SEM) reported.

Table 2. Effect of forage specie, N fertilization and harvest date on in-vitro organic matter digestibility in tropical grasses[a,b]

Species	Harvest[d,e]	N fertilization rates[c], kg/(ha·cutting) 0	39	79	118	157	Mean (harvest)	SEM[f]
Bahiagrass	1	53.1	53.2	53.4	55.3	55.8	54.2	1.14
	2	53.9	54.5	52.5	53.4	54.9	53.8	1.14
	3	50.6	48.5	47.8	48.4	48.6	48.8	1.14
	4	52.5	51.2	50.8	51.1	50.3	51.2	1.20
	5	50.2	51.1	51.4	51.4	53.2	51.5	1.14
Mean (fertilization)		52.1	51.7	51.2	51.9	52.6		
Bermudagrass	1	60.4	59.1	59.6	61.6	64.5	61.0	1.14
	2	61.0	57.8	57.2	57.2	59.9	58.6	1.14
	3	55.2	53.7	53.3	53.0	56.4	54.3	1.14
	4	56.4	56.4	56.6	58.0	59.4	57.4	1.14
	5	57.1	54.3	53.5	56.1	59.9	56.2	1.14
Mean (fertilization)		58.0	56.3	56.0	57.2	60.0		
Stargrass	1	54.0	56.6	59.8	61.2	61.5	58.6	1.14
	2	51.1	53.3	52.9	55.4	56.9	53.9	1.14
	3	51.3	50.0	52.3	54.6	56.3	52.9	1.14
	4	51.5	52.1	56.1	56.7	57.7	54.8	1.14
	5	50.7	51.6	52.0	56.3	55.5	53.2	1.14
Mean (fertilization)		51.7	52.7	54.6	56.8	57.6		

[a]LS means reported, % of forage DM.
[b]Probability for effects in the model: fertilization, $P < .01$; species, $P < .01$; harvest date, $P < .01$; fertilization*species, $P < .01$; fertilization*harvest date $P < .05$; species*harvest date, $P < .01$.
[c]Fertilization by species: bahiagrass, no effect ; bermudagrass, quadratic ($P < .01$); stargrass, linear ($P < .01$)
[d]Quadratic effect of harvest across all species and fertilization rates, P<.01.
[e]Harvest 1, early June; harvest 2, late June; harvest 3, July; harvest 4, August; harvest 5, September.
[f]Greatest standard error of treatment means (SEM) reported.

Table 3. Effect of forage specie, N fertilization and harvest date on percentage of ADF in tropical grasses[a,b]

Species	Harvest[d,e]	N fertilization rates[c], kg/(ha·cutting)					Mean	
		0	39	79	118	157	(harvest)	SEM[f]
Bahiagrass	1	34.2	34.4	33.8	33.2	32.8	33.7	.75
	2	35.3	35.6	34.8	35.4	33.4	34.9	.75
	3	37.9	37.9	37.1	36.0	37.7	37.3	.70
	4	36.5	36.5	38.4	36.2	40.3	37.6	.70
	5	37.2	36.0	35.6	35.0	34.8	35.7	.75
Mean (fertilization)		36.2	36.1	35.9	35.2	35.8		
Bermudagrass	1	31.6	31.3	32.7	31.1	30.7	31.5	.70
	2	32.5	33.4	33.8	33.6	32.9	33.2	.70
	3	34.1	35.7	35.2	34.8	34.4	34.8	.70
	4	33.5	35.1	34.8	34.3	33.1	34.2	.70
	5	33.4	35.3	35.0	33.3	33.0	34.0	.75
Mean (fertilization)		33.0	34.2	34.3	33.4	32.8		
Stargrass	1	31.0	30.5	32.9	30.4	29.8	30.9	.70
	2	33.1	33.4	33.7	32.9	32.1	33.0	.70
	3	33.6	34.1	33.3	32.8	32.1	33.1	.75
	4	34.2	34.9	34.5	33.3	32.4	33.9	.75
	5	34.0	34.1	33.7	32.3	32.3	33.3	.70
Mean (fertilization)		33.2	33.4	33.6	32.3	31.7		

[a]LS means reported, % of forage DM.

[b]Probability for effects in the model: fertilization, $P < .01$; species, $P < .01$; harvest date, $P < .01$; fertilization*species, $P < .08$; fertilization*harvest date $P < .01$; species*harvest date, $P < .01$.

[c]Fertilization by species: bahiagrass, no effect; bermudagrass, quadratic ($P < .01$); stargrass, quadratic ($P < .05$)

[d]Quadratic effect of harvest, across all species and fertilization rates, $P < .01$.

[e]Harvest 1, early June; harvest 2, late June; harvest 3, July; harvest 4, August; harvest 5, September.

[f]Greatest standard error of treatment means (SEM) reported.

Table 4. Effect of forage specie, N fertilization and harvest date on percentage of NDF in tropical grasses[a,b]

Species	Harvest[d,e]	N fertilization rates[c], kg/(ha·cutting)					Mean	
		0	39	79	118	157	(harvest)	SEM[f]
Bahiagrass	1	75.6	74.7	73.8	73.9	72.5	74.1	.96
	2	75.6	75.4	74.9	73.1	74.2	74.6	.96
	3	75.3	76.0	75.3	74.1	73.0	74.7	.96
	4	75.8	77.9	76.2	74.9	74.7	75.9	.96
	5	78.0	77.2	76.2	77.6	75.3	76.9	.96
Mean (fertilization)		76.1	76.2	75.3	74.7	73.9		
Bermudagrass	1	76.0	75.9	75.0	73.9	71.9	74.5	.96
	2	77.4	79.5	77.6	77.6	76.0	77.6	.96
	3	78.4	78.7	77.1	77.2	76.9	77.7	1.02
	4	77.5	79.3	77.9	77.8	77.3	78.0	.96
	5	79.2	79.3	79.6	79.6	76.3	78.8	.96
Mean (fertilization)		77.7	78.5	77.4	77.2	75.7		
Stargrass	1	74.7	72.9	71.2	70.0	70.5	71.9	.96
	2	78.3	77.2	74.6	73.3	73.8	75.4	.96
	3	77.2	76.3	73.9	72.4	71.6	74.3	.96
	4	76.1	76.3	74.0	72.7	70.5	73.9	.96
	5	78.2	76.8	75.8	73.5	73.4	75.5	.96
Mean (fertilization)		76.9	75.9	73.9	72.4	72.0		

[a]LS means reported, % of forage DM.
[b]Probability for effects in the model: fertilization, $P < .01$; species, $P < .01$; harvest date, $P < .01$; fertilization*species, $P < .05$; fertilization*harvest date $P = .61$; species*harvest date, $P < .01$.
[c]Linear effect of fertilization, across all species and harvest dates, $P < .01$.
[d]Harvest date by species: bahiagrass, linear ($P < .01$); bermudagrass, quadratic ($P < .05$); stargrass, cubic ($P < .01$).
[e]Harvest 1, early June; harvest 2, late June; harvest 3, July; harvest 4, August; harvest 5, September.
[f]Greatest standard error of treatment means (SEM) reported.

Table 5. Effect of forage specie, N fertilization and harvest date on percentage of total Nitrogen in tropical grasses[a,b]

Species	Harvest[d,e]	N fertilization rates[c], kg/(ha·cutting)					Mean	
		0	39	79	118	157	(harvest)	SEM[f]
Bahiagrass	1	1.48	1.76	2.00	2.17	2.50	1.98	.07
	2	1.34	1.57	1.86	2.17	2.29	1.85	.07
	3	1.39	1.65	1.95	2.27	2.36	1.92	.07
	4	1.54	1.63	1.94	2.15	2.34	1.92	.07
	5	1.46	1.78	2.27	2.50	2.70	2.14	.07
Mean (fertilization)		1.44	1.68	2.00	2.25	2.44		
Bermudagrass	1	1.70	1.95	2.29	2.66	3.12	2.34	.07
	2	1.55	1.69	2.11	2.38	2.82	2.11	.07
	3	1.45	1.56	2.09	2.23	2.57	1.98	.07
	4	1.51	1.61	2.21	2.49	2.80	2.12	.07
	5	1.66	1.93	2.33	2.84	3.17	2.39	.07
Mean (fertilization)		1.57	1.75	2.21	2.52	2.90		
Stargrass	1	1.89	2.21	2.68	3.04	3.20	2.60	.07
	2	1.67	1.87	2.27	2.84	3.00	2.33	.07
	3	1.55	1.69	2.14	2.71	2.81	2.18	.07
	4	1.43	1.84	2.14	2.63	3.08	2.22	.07
	5	1.63	2.02	2.61	3.12	3.49	2.57	.07
Mean (fertilization)		1.63	1.93	2.37	2.87	3.12		

[a]LS means reported, % of forage DM.

[b]Probability for effects in the model: fertilization, $P < .01$; species, $P < .01$; harvest date, $P < .01$; fertilization*species, $P < .01$; fertilization*harvest date $P < .01$; species*harvest date, $P < .01$.

[c]Fertilization by species: bahiagrass, linear ($P < .01$); bermudagrass, linear ($P < .01$); stargrass, cubic ($P < .05$).

[d]Quadratic effect of harvest, across all species and fertilization rates, P<.01.

[e]Harvest 1, early June; harvest 2, late June; harvest 3, July; harvest 4, August; harvest 5, September.

[f]Greatest standard error of treatment means (SEM) reported.

Table 6. Effect of specie and fertilization on protein fractions in tropical grasses, across all harvest dates [a]

Specie	Fraction	N fertilization rates[c], kg/(ha·cutting)					Fraction	
		0	39	79	118	157	Means	SEM[b]
Bahiagrass								
	A	.31^{c,x}	.37^{c,x}	.51^{d,x}	.56^{d,x}	.70^{e,x}	.49	.03
	B_1	.13^{c,x}	.14^{c,x}	.18^{cd,x}	.22^{d,x}	.22^{d,x}	.18	.02
	B_2	.17^{c,x}	.19^{cd,x}	.21^{cd,x}	.23^{de,x}	.25^{e,x}	.21	.02
	B_3	.67^{c,x}	.76^{d,x}	.86^{e,x}	1.00^{f,x}	.99^{f,x}	.86	.03
	C	.18^{c,x}	.23^{d,x}	.27^{e,x}	.28^{e,x}	.30^{f,x}	.25	.01
	Total	1.46	1.69	2.03	2.29	2.46		
Bermudagrass								
	A	.49^{c,y}	.55^{c,y}	.82^{d,y}	.95^{e,y}	1.16^{f,y}	.79	.03
	B_1	.13^{c,x}	.15^{cd,x}	.19^{de,x}	.21^{ef,x}	.24^{f,x}	.118	.02
	B_2	.21^{c,x}	.28^{d,y}	.31^{d,y}	.40^{e,y}	.44^{e,y}	.33	.02
	B_3	.62^{c,x}	.63^{c,y}	.72^{d,y}	.77^{d,y}	.86^{e,y}	.72	.03
	C	.13^{c,y}	.15^{d,y}	.17^{e,y}	.19^{f,y}	.20^{f,y}	.17	.01
	Total	1.58	1.76	2.21	2.52	2.90		
Stargrass								
	A	.46^{c,y}	.67^{d,z}	.82^{e,y}	1.17^{f,z}	1.31^{g,z}	.89	.03
	B_1	.18^{c,y}	.17^{c,x}	.25^{d,y}	.28^{de,y}	.31^{e,y}	.24	.02
	B_2	.21^{c,x}	.29^{d,y}	.38^{e,z}	.42^{ef,y}	.47^{f,y}	.35	.02
	B_3	.66^{cd,x}	.64^{c,y}	.74^{de,y}	.80^{ef,y}	.82^{f,y}	.73	.03
	C	.14^{c,y}	.16^{d,y}	.17^{e,y}	.21^{f,y}	.20^{ef,y}	.18	.01
	Total	1.65	1.93	2.36	2.88	3.11		

[a]All fraction LS means expressed as a percentage of forage dry matter.
[b]Greatest standard error of treatment means (SEM) reported.
[cdefg]*Means within a row lacking a common superscript letter differ* ($P < .05$).
[xyz]For each fraction (A, B_1, B_2, B_3, C), across all species, means within a column lacking a common superscript differ ($P < .05$).

Table 7. Effect of specie and harvest date on protein fractions in tropical grasses, across all fertilization rates[a]

Specie	Fraction	N fertilization rates[c], kg/(ha·cutting)					Fraction	
		0	39	79	118	157	Means	SEM[b]
Bahiagrass								
	A	$.53^{d,x}$	$.52^{d,x}$	$.43^{e,x}$	$.41^{e,x}$	$.57^{d,x}$	.51	.03
	B_1	$.16^{d,x}$	$.13^{d,x}$	$.24^{e,x}$	$.17^{d,xy}$	$.18^{d,x}$	.18	.02
	B_2	$.26^{df,x}$	$.18^{deg,x}$	$.13^{g,x}$	$.23^{de,x}$	$.26^{d,x}$	.21	.03
	B_3	$.84^{d,x}$	$.85^{d,x}$	$.85^{d,x}$	$.84^{d,x}$	$.89^{d,x}$	.85	.03
	C	$.20^{d,x}$	$.24^{e,x}$	$.31^{f,x}$	$.28^{g,x}$	$.24^{e,x}$	.25	.01
	Total	1.99	1.92	1.93	1.90	2.14		
Bermudagrass								
	A	$.82^{df,y}$	$.78^{d,y}$	$.70^{e,y}$	$.78^{d,y}$	$.89^{f,y}$	.79	.03
	B_1	$.26^{d,y}$	$.14^{e,xy}$	$.18^{e,y}$	$.16^{e,x}$	$.16^{e,x}$	.18	.02
	B_2	$.33^{d,x}$	$.26^{d,y}$	$.26^{d,y}$	$.34^{d,y}$	$.45^{e,y}$	.33	.03
	B_3	$.78^{d,x}$	$.78^{d,x}$	$.66^{e,y}$	$.66^{e,y}$	$.72^{de,y}$	.72	.03
	C	$.15^{d,y}$	$.16^{de,y}$	$.18^{e,y}$	$.18^{e,y}$	$.17^{e,y}$	.17	.01
	Total	2.34	2.12	1.98	2.12	2.39		
Stargrass								
	A	$.93^{d,z}$	$.87^{de,z}$	$.79^{e,z}$	$.81^{e,y}$	$1.01^{f,z}$	.88	.03
	B_1	$.34^{d,z}$	$.19^{e,y}$	$.24^{e,x}$	$.22^{e,y}$	$.20^{e,x}$	.24	.02
	B_2	$.38^{df,x}$	$.26^{e,y}$	$.29^{de,y}$	$.40^{f,y}$	$.45^{f,y}$	.36	.03
	B_3	$.79^{df,x}$	$.84^{de,x}$	$.68^{gh,y}$	$.61^{h,y}$	$.74^{efg,y}$	.73	.03
	C	$.16^{d,y}$	$.20^{e,z}$	$.18^{d,y}$	$.18^{d,y}$	$.18^{d,y}$	.18	.01
	Total	2.60	2.36	2.18	2.22	2.58		

[a]All fraction LS means expressed as a percentage of forage dry matter.

[b]Harvest 1, early June; harvest 2, late June; harvest 3, July; harvest 4, August; harvest 5, September.

[c]Greatest standard error of treatment means (SEM) reported.

[defgh]Means within a row lacking a common superscript letter differ ($P<.05$).

[xyz]For each fraction (A, B_1, B_2, B_3, C), across all species, means within a column lacking a common superscript differ ($P < .05$).

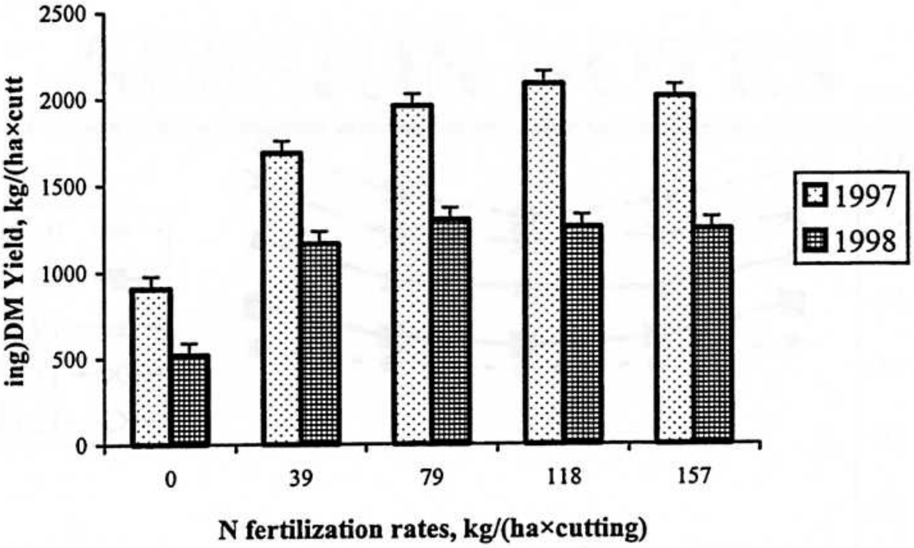

Figure 1. Effects of N fertilization and year on forage DM yield (LS means) of tropical grasses (fertilization x year; $P < .01$)

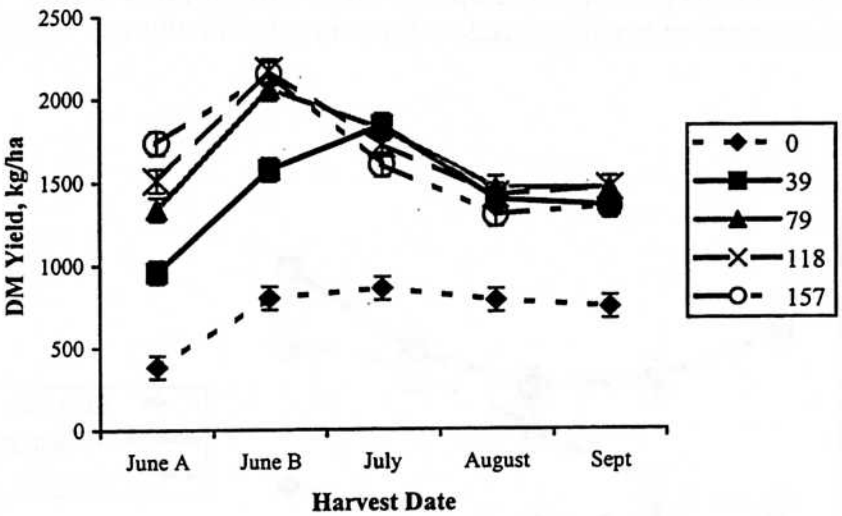

Figure 2. Effects of harvest date and N fertilization (kg N/(ha·cutting)) on forage DM yields (LS means) of tropical grasses (harvest date x fertilization; $P < .01$)

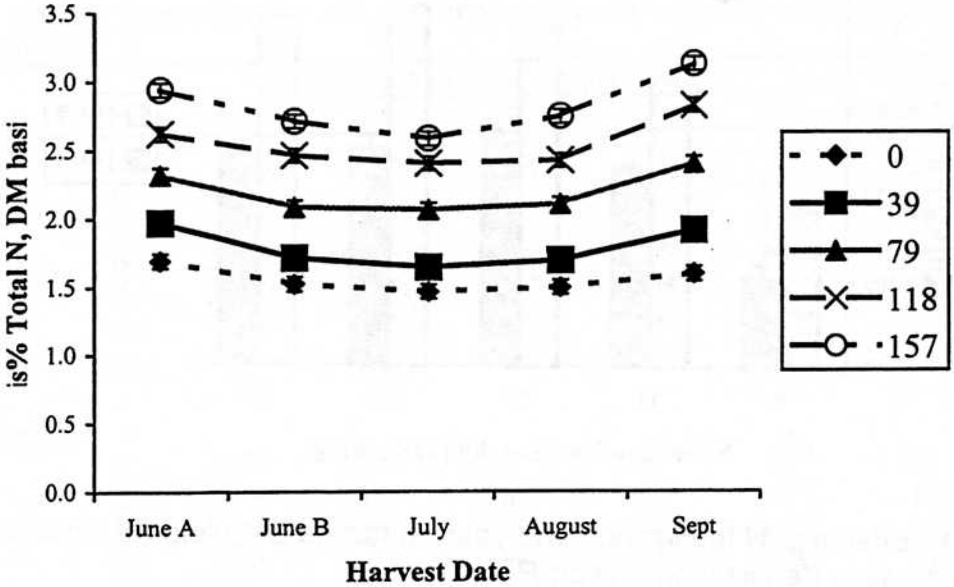

Figure 3. Effects of N fertilization and specie on in-vitro organic matter digestibility (LS means) of tropical grasses (specie x fertilization; $P < .01$)

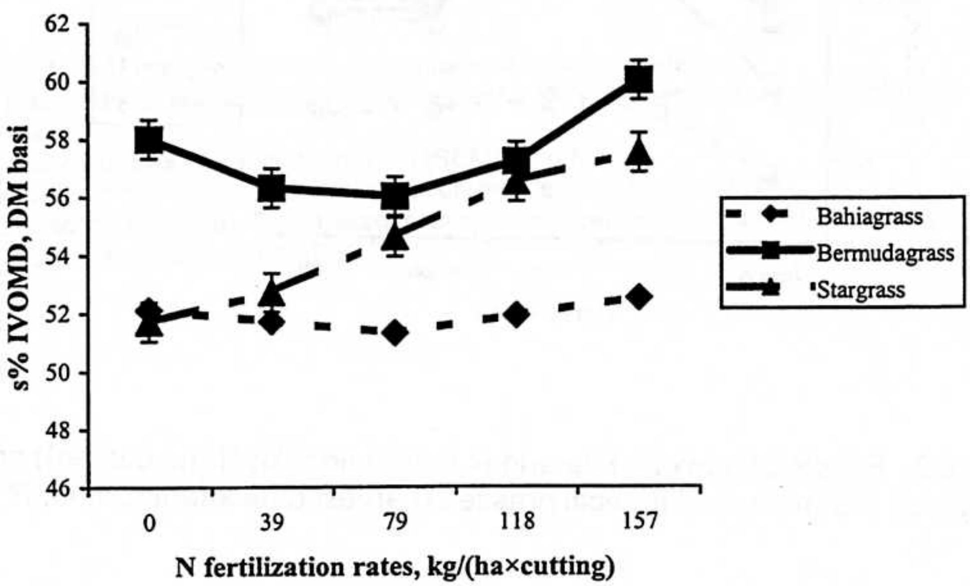

Figure 4. Effects of harvest date and N fertilization (kg N/(ha·cutting)) on total N (LS means) of tropical grasses (harvest date x fertilization; $P < .01$)