Nutrient Content of Dehydrated Coastal Bermuda Grass and Pearl Millet

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Dehydrated coastal Bermuda grass and pearl millet were analyzed for proximate constituents, carbohydrate fractions, carotenoid pigments, vitamins, minerals, amino acids, and nitrates. Coastal Bermuda grass, compared to pearl millet of similar age, contained less ash, protein, cell contents, protein in doubly extracted residue, carotene, and xanthophyll; more NFE, lignin, other carbohydrates, acid-detergent fiber, cell wall constituents, doubly extracted residue, and protein-free doubly extracted residue; and the same ether extract, crude fiber, and cellulose. Coastal Bermuda grass had more thiamin, niacin, biotin, and choline, less pantothenic acid, and similar amounts of riboflavin, pyridoxine, and folic acid. The ash of coastal Bermuda grass showed more Na, P, and Zn and similar amounts of K, Ca, Mg, and Mn. Coastal Bermuda grass consisted of more isoleucine, leucine, and methionine, less histidine, and similar amounts of the other amino acids. Coastal Bermuda grass had less nitrate. The data should provide the basis for the use of similar products as xanthophyll sources in poultry feeds.

Dehydrated coastal Bermuda grass and pearl millet represent potential xanthophyll sources for commercial poultry feeds (Wilkinson and Barbee, 1968 a,b). Coastal Bermuda grass is a perennial sterile hybrid characterized by upright growth and high leaf-to-stem ratio (Burton, 1954). It is estimated that 6,000,000 acres of this grass are grown in the southern and southeastern United States. Pearl millet is a high yielding summer annual plant adaptable to the same area. There is a paucity of composition data for high quality dehydrated coastal Bermuda grass products and, to the authors' knowledge, no data for dehydrated pearl millet products. The work reported herein was designed to provide useful linear programming data on the content of certain major nutrients, carotenoid pigments, vitamins, minerals, amino acids, and nitrates in such products.

MATERIALS AND METHODS

The forages were grown in 1964, 1965, and 1966 on Tifton loamy sand. Portions of a uniform, well established coastal Bermuda grass sod were cut at 21-, 24- to 25-, 28-, and 35-day intervals throughout one or more growing seasons. Gahi I hybrid of pearl millet was seeded at about 30 pounds per acre and portions of the uniform field were cut at 21-, 26- to 29-, 31- to 35-, and 39- to 45-day intervals throughout one or more growing seasons. At the beginning of the season and after each cutting, equal amounts of N were applied to appropriate areas to give about 600 pounds per acre over the growing season. P and K were applied at three equally spaced intervals in amounts necessary to give a 4:1:2 ratio of $N:P_2O_5:K_2O$ for the season.

Forage was harvested, artificially dehydrated, and pelleted by commercial methods. Pellets were ground in a Wiley mill to pass a 20-mesh screen before analysis. Analyses were completed as quickly as possible after production, but pellets were held in polyethylene bags inside sealed metal cans at 4° C. before removal for grinding and analysis.

Proximate analysis was performed according to the Association of Official Agricultural Chemists procedure (1965). Water and grit were determined for each sample, and the content of other constituents was then expressed on a moisture-free, grit-free basis. Certain other methods of partitioning digestible and indigestible fractions were also employed. Cellulose, lignin, and other carbohydrates were determined according to Crampton and Maynard (1938), except that lignin was determined by the method of Davis and Miller (1939). Acid-detergent fiber was analyzed by the method of Van Soest (1963); cell wall constituents and cell contents by the procedure of Van Soest and Marcus (1964); and doubly extracted residue fractions according to Binger *et al.* (1961). Carotene and xanthophyll were determined in reground, freshly processed samples by the method of Kohler *et al.* (1967).

Randomly selected samples were analyzed for thiamin, riboflavin, pyridoxine, niacin, panthothenic acid, choline, folic acid, and biotin by the Wisconsin Alumni Research Foundation Laboratories, Madison. Thiamin, riboflavin, niacin, and folic acid were determined by AOAC (1965) procedures. Pyridoxine, pantothenic acid, choline, and

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biotin assays employed the methods of Atkins *et al.* (1943), Nielands and Strong (1948), Horowitz and Beadle (1943), and Wright and Skeggs (1944), respectively. Results were expressed as milligrams per kilogram of dry matter.

Randomly selected samples were dried and then ashed by the ternary acid wet oxidation procedure of Johnson and Ulrich (1959). Ca, Mg, Na, K, Zn, and Mn were determined with a Perkin-Elmer Model 303 atomic absorption spectrophotometer using commercial standards. P was determined by the colorimetric molybdenum blue method, using stannous chloride as a reducing agent (Johnson and Ulrich, 1959). Results were expressed as percentage of the ash.

Randomly selected samples were hydrolyzed for 24 and 72 hours with 6*N* HCl under reflux at 140° C. and analyzed for amino acids by the method of Moore *et al.* (1958), except that the colorimetric ninhydrin analysis according to Rosen (1957) and Grant (1963) was employed. Cysteic acid was determined in two samples of dehydrated pearl millet and one sample of coastal Bermuda grass following performic acid oxidation as described by Moore (1963) and hydrolysis with 6*N* HCl at 140° C. for 22 hours. All hydrolyzates were analyzed for micro-Kjeldahl N by the AOAC (1965) procedure and amino acid content expressed as grams per 16 grams of N, using the formula of Hirs *et al.* (1954) to extrapolate to zero time where appropriate.

The colorimetric brucine method for nitrate described by the American Public Health Association (1960) was employed with hot water extracts which had been treated with the copper-silver-$Ca(OH)_2$ clarifying reagent of Harper (1924) plus nitrate-free decolorizing charcoal. Special care should be observed in the use of the corrosive and toxic H_2SO_4-brucine reagent. The nitrate content was expressed as per cent KNO_3 (dry matter basis).

RESULTS AND DISCUSSION

The results are given as mean plus or minus standard error values in Tables I, II, and III. Certain analyses, inadvertently omitted, are indicated by n.d. (not determined).

The proximate analysis (Table I) shows that coastal Bermuda grass, compared with pearl millet of comparable age, contained 64 and 77% as much ash and protein, respectively, 18% more nitrogen-free extract (NFE), and similar amounts of crude fiber and ether extract. The proximate analysis scheme, required by today's feed laws, has questionable validity in predicting nutritional value. The use of crude fiber and NFE to differentiate indigestible and digestible fractions is known to be erroneous. Norman (1935) showed that lignin, which is indigestible, is largely solubilized in hot alkali and thus included in the NFE fraction of the proximate analysis scheme. Several other measures of digestible and indigestible fractions of feedstuffs have subsequently been proposed, and data for several of these are included in Table I. The significance and relationship of some of these measures are discussed by Van Soest (1965, 1966) and Kim *et al.* (1967). Since coastal Bermuda grass, compared with pearl millet of comparable age, has a higher content of lignin, acid-detergent fiber, and cell wall constituents, it should be less digestible.

The doubly extracted residue system was used by Binger *et al.* (1961) to study dehydrated alfalfa extensively. Comparisons of their data with those in Table I show that coastal Bermuda grass and pearl millet contain more doubly extracted residue and protein-free doubly extracted residue than alfalfa but similar amounts of protein in the doubly extracted residue. Binger *et al.* found protein-free doubly extracted residue to be closely related to total cell wall material in dehydrated alfalfa and found lignin to approach a constant 20% of protein-free doubly extracted residue. Protein-free doubly extracted residue for coastal Bermuda grass and pearl millet was consistently lower than the cell wall constituents fraction, and lignin approached a constant 14% of protein-free doubly extracted residue. Van Soest (1966) mentioned this difference in the relation-

Table I. Percentage of Proximate Components and Carbohydrate Fractions and Milligrams per Kilogram of Carotene and Xanthophyll in Dehydrated Grasses of Various Ages

	Coastal Bermuda Grass				Pearl Millet			
Age, days	21	24–25	28	35	21	26–29	31–35	39
Number tested	8	12	6	5	6	7	8	3
Ash	5.8 ± 0.9[a]	5.7 ± 0.5	5.8 ± 0.3	5.6 ± 0.4	8.5 ± 0.6	9.2 ± 0.8	9.0 ± 0.6	10.7 ± 0.4
Crude protein	18.1 ± 3.1	17.7 ± 1.5	16.2 ± 1.7	14.1 ± 2.4	22.4 ± 1.5	20.5 ± 1.2	19.7 ± 1.2	19.2 ± 0.7
Ether extract	5.4 ± 0.8	5.9 ± 0.9	4.8 ± 0.5	3.9 ± 0.5	6.0 ± 0.3	5.5 ± 0.3	5.4 ± 0.4	4.0 ± 0.2
Cellulose	27.9 ± 1.3	26.7 ± 1.6	28.4 ± 1.8	29.4 ± 0.9	26.2 ± 1.0	27.8 ± 0.8	28.4 ± 0.8	n.d.
Lignin	7.9 ± 0.8	8.8 ± 0.5	8.0 ± 0.7	9.1 ± 1.0	6.6 ± 0.4	7.4 ± 0.2	7.9 ± 0.3	8.7 ± 0.3
Other carbohydrates	34.9 ± 4.8	35.1 ± 2.3	36.7 ± 2.9	38.0 ± 2.2	34.2 ± 1.1	32.8 ± 1.0	31.1 ± 2.4	n.d.
Acid-detergent fiber	32.4 ± 2.1	31.4 ± 2.3	31.8 ± 1.5	33.6 ± 0.7	29.9 ± 1.8	31.5 ± 1.3	32.0 ± 1.1	n.d.
Cell wall constituents	68.6 ± 2.4	67.7 ± 1.9	70.0 ± 1.6	72.9 ± 2.6	56.7 ± 2.5	59.0 ± 1.3	59.2 ± 1.1	64.4 ± 1.4
Cell contents	31.4 ± 2.4	32.3 ± 1.9	30.0 ± 1.6	27.1 ± 2.6	43.3 ± 2.5	41.0 ± 1.3	40.8 ± 1.1	35.6 ± 1.4
Doubly extracted residue	72.7 ± 1.6	72.5 ± 1.9	72.8 ± 1.8	72.3 ± 2.6	65.2 ± 3.2	66.0 ± 1.9	65.1 ± 2.3	70.4 ± 1.0
Protein in doubly extracted residue	12.6 ± 2.0	12.6 ± 1.0	10.9 ± 1.6	10.2 ± 1.2	14.0 ± 2.1	12.1 ± 1.7	11.1 ± 2.0	10.6 ± 0.7
Protein-free doubly extracted residue	60.1 ± 3.0	59.9 ± 2.8	61.9 ± 2.4	62.1 ± 3.4	51.2 ± 2.2	53.9 ± 3.5	54.0 ± 3.8	59.8 ± 1.7
Carotene	358 ± 80.9	391 ± 49.2	338 ± 47.6	262 ± 55.7	427 ± 33.8	340 ± 46.5	339 ± 75.3	n.d.
Xanthophyll	523 ± 125.0	561 ± 80.6	474 ± 50.2	383 ± 70.0	566 ± 100.1	458 ± 100.5	457 ± 126.6	n.d.

[a] Mean ± standard error.

Table II. Vitamin and Mineral Content of Dehydrated Grasses

Vitamins, Mg./Kg.	Coastal Bermuda Grass	Pearl Millet
	Number Tested	
	12	10
Thiamin	4.29 ± 0.15[a]	1.22 ± 0.20
Riboflavin	10.76 ± 0.16	12.55 ± 0.58
Niacin	75.83 ± 1.02	50.58 ± 2.40
Pyridoxine	7.96 ± 0.27	8.23 ± 0.48
Pantothenic acid	12.28 ± 0.65	19.27 ± 1.60
Folic acid	1.38 ± 0.07	1.48 ± 0.27
Biotin	0.34 ± 0.06	0.17 ± 0.02
Choline	1968 ± 40	1645 ± 209
Minerals, % of Ash	**Number Tested**	
	30	**24**
Na	1.28 ± 0.24	0.60 ± 0.04
K	38.06 ± 0.91	36.04 ± 0.88
Ca	3.88 ± 0.89	3.48 ± 0.30
Mg	3.88 ± 0.13	3.75 ± 0.19
P	3.87 ± 0.14	2.63 ± 0.09
Zn	0.59 ± 0.08	0.20 ± 0.03
Mn	0.18 ± 0.01	0.15 ± 0.01

[a] Mean ± standard error.

Table III. Amino Acid and Nitrate Content of Dehydrated Grasses

Amino Acids, G./16 G. N	Number Tested	
	5	8
Alanine	6.2 ± 0.5[a]	5.7 ± 0.3
Arginine	3.6 ± 0.2	4.3 ± 0.4
Aspartic acid	12.7 ± 0.9	11.0 ± 0.6
Cystine	0.9	1.0
Glycine	4.3 ± 0.3	4.9 ± 0.3
Glutamic acid	9.8 ± 0.6	9.5 ± 0.4
Histidine	1.7 ± 0.1	2.3 ± 0.3
Hydroxyproline	20.5 ± 2.7	21.2 ± 2.1
Isoleucine	4.3 ± 0.3	3.7 ± 0.2
Leucine	8.4 ± 0.6	7.2 ± 0.4
Lysine	4.3 ± 0.2	4.2 ± 0.4
Methionine	1.2 ± 0.1	0.9 ± 0.1
Phenylalanine	4.0 ± 0.3	3.9 ± 0.3
Proline	3.1 ± 0.2	3.1 ± 0.3
Serine	3.7 ± 0.6	4.3 ± 0.5
Threonine	3.3 ± 0.3	4.0 ± 0.5
Tyrosine	2.7 ± 0.3	2.9 ± 0.5
Tryptophan	n.d.	n.d.
Valine	5.4 ± 0.4	4.9 ± 0.3
Total	100.1	99.0
	Number Tested	
Nitrate	**15**	**9**
KNO_3, % of dry matter	0.7 ± 0.1	3.5 ± 0.3

[a] Mean ± standard error.

ship of cell wall and lignin between alfalfa and the grasses and used this as an argument to show why no single measure can adequately classify the nonnutritive portion of all feedstuffs. He was reluctant to recommend adoption of any new system to replace proximate analysis but recommended continued study of alternative systems. For this reason data for several possible alternatives to proximate analysis are given in Table I.

The content of carotene and xanthophyll in both grasses declined with increasing age, but could be modified somewhat by environmental conditions. For instance, the highest coastal Bermuda grass values found at 24 to 25 days probably reflect more uniform rainfall during the season. While pearl millet tended to have more carotene and xanthophyll at a given age than coastal Bermuda grass, both may be considered excellent in carotenoid content at all ages studied, and the selection of cutting frequency for commercial dehydration must be made on other factor(s) such as yield, cost of processing, or protein content. The method employed for xanthophyll analysis is closely correlated with broiler pigmentation (Kuzmicky *et al.*, 1968; Wilkinson and Barbee, 1968a).

Coastal Bermuda grass contained 352, 150, 200, and 120% as much thiamin, niacin, biotin, and choline, respectively, but only 64% as much pantothenic acid as pearl millet (Table II). The two grasses were similar in riboflavin, pyridoxine, and folic acid content.

The ash of coastal Bermuda grass contained 213, 147, and 295% as much Na, P, and Zn, respectively, as pearl millet and similar quantities of K, Ca, Mg, and Mn (Table II). However, coastal Bermuda grass contained only 64% as much ash as pearl millet.

Coastal Bermuda grass contained 116, 117, and 133% as much isoleucine, leucine, and methionine, respectively, but only 74% as much histidine as pearl millet (Table III). The two grasses were similar in their content of other amino acids. The S-containing amino acids, methionine and cystine, are most limiting in these products, as they are in plant proteins in general. Tryptophan was not determined because of difficulties in quantitative hydrolysis and separation (Robel, 1967; Spies, 1967). An interesting and hitherto unreported finding is the very high (20%) hydroxyproline content in these materials. Essentially all the hydroxyproline in animal tissues is found in collagen (Kivirikko *et al.*, 1967), and further work with plant material should be done to determine the relationship, if any, of this amino acid to the indigestible matter in plants.

Nitrate poisoning of livestock from hay, silage, or green forage containing excess nitrate has been established for many years. McCreery *et al.* (1966) found Gahi millet to have toxic levels of nitrate when highly fertilized and harvested at frequent intervals. This prompted determinations in dehydrated coastal Bermuda grass and pearl millet (Table III), which showed the former to be well below and the latter well above the accepted toxic level (1% KNO_3, dry matter basis). This could be a serious deterrent in the use of pearl millet for dehydration, if the product was to be used at levels above 30% in the total ration. So high a level would not normally be employed in poultry feeds. Thus, nitrate content should not prevent the commercial production of dehydrated millet for this purpose, if otherwise economically feasible.

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