

pears to be well recognized and adequately controlled by means of the inspection process.

The information obtained in this study shows that plants can vary greatly in their PA content. PA levels probably rise in response to stress conditions such as drought and infertile soils. These data indicate that grazing of *Senecio* species should be particularly avoided during the bud and flower stages and that *Crotalaria* is most hazardous during seed stages. The insidious nature of pyrrolizidine alkaloid toxicosis, the great variability of alkaloid content, and the poor understanding of the relative toxicities and metabolism of the free base and *N*-oxide forms dictate that the utmost effort should be made to control access of grazing animals to PA-producing plants at all times and to prevent entry of PAs into the human food chain.

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LITERATURE CITED

- Adams, R.; Rogers, E. F. *J. Am. Chem. Soc.* **1939**, *61*, 2815.
 Barkley, T. M. *North Am. Flora II* **1978**, *10*, 50.
 Bull, L. B.; Culvenor, C. C. J.; Dick, A. T. "The Pyrrolizidine Alkaloids"; North-Holland Publishing Co.: Amsterdam, 1968.
 Culvenor, C. C. J.; Clarke, M.; Edgar, J. A.; Frahn, J. L.; Jago, M. V.; Peterson, J. E.; Smith, L. W. *Experientia* **1980**, *36*, 377.
 Culvenor, C. C. J.; Edgar, J. A.; Smith, L. W. *J. Agric. Food Chem.* **1981**, *29*, 958.
 Culvenor, C. C. J.; Smith, L. W. *Aust. J. Chem.* **1966**, *19*, 1955.
 Deinzer, M. L.; Arbogast, B. L.; Buhler, D. R.; Cheeke, P. R. *Anal. Chem.* **1982**, *54*, 1811.
 Deinzer, M. L.; Thomson, P. A.; Burgett, D. M.; Isaacson, D. L. *Science (Washington, D.C.)* **1977**, *195*, 497.
 Dickinson, J. O.; Cooke, M. P.; King, R. R.; Mohamed, P. A. *J. Am. Vet. Med. Assoc.* **1976**, *169*, 1192.
 Johnson, A. E. *Am. J. Vet. Res.* **1978**, *39*, 1542.
 Johnson, A. E. In "Symposium on Pyrrolizidine (*Senecio*) Alkaloids: Toxicity, Metabolism and Poisonous Plant Control Measures"; Cheeke, P. R., Ed.; The Nutrition Research Institute: Corvallis, OR, 1979; pp 129-134.
 Johnson, A. E.; Molyneux, R. J. *Am. J. Vet. Res.* **1984**, *45*, 26.
 Johnson, A. E.; Molyneux, R. J.; Stuart, L. D. *Am. J. Vet. Res.* **1984**, in press.
 Kumari, S.; Kapur, K. K.; Atal, C. K. *Curr. Sci.* **1966**, *35*, 546.
 Mathews, F. P. *Tex., Agric. Exp. Stn., [Bull.]* **1933**, *481*, 1.
 Mattox, A. R. In "Effects of Poisonous Plants on Livestock", Keeler, R. F.; Van Kampen, K. R.; James, L. F., Eds.; Academic Press: New York, 1978; pp 177-187.
 McCulloch, E. C. *J. Am. Vet. Med. Assoc.* **1940**, *96*, 5.
 McLean, E. K. *Pharmacol. Rev.* **1970**, *22*, 429.
 Molyneux, R. J.; Johnson, A. E.; Roitman, J. N.; Benson, M. E. *J. Agric. Food Chem.* **1979**, *27*, 494.
 Roitman, J. N. *Lancet* **1981**, *i*, 944.
 Roitman, J. N.; Molyneux, R. J.; Johnson, A. E. In "Symposium on Pyrrolizidine (*Senecio*) Alkaloids: Toxicity, Metabolism and Poisonous Plant Control Measures"; Cheeke, P. R., Ed.; The Nutrition Research Institute: Corvallis, OR, 1979; pp 23-33.
 Sipple, W. L. *Ann. N.Y. Acad. Sci.* **1964**, *3*, 562.
 Smith, L. W.; Culvenor, C. C. J. *J. Nat. Prod.* **1981**, *44*, 129.
 Snyder, S. P. *Oreg. Cattleman* **1972**, *21*, 22.
 Stillman, A. E.; Huxtable, R.; Consroe, P.; Kohonen, P.; Smith, S. *Gastroenterology* **1977**, *73*, 349.
 Van Es, L.; Cantwell, L. R.; Martin, H. M.; Kramer, J. *Univ. Nebr. Agric. Exp. Res. Bull.* **1929**, *43*, 1.

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Studies on Seed Protein of Pearl Millets. 1. Amino Acid Composition of Protein Fractions of Early and Late Maturing Varieties

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Grain proteins of seven varieties of early and late season pearl millets (*Pennisetum typhoides*) were separated into five soluble fractions by the Landry-Moureaux method. The distribution of protein among the five fractions was identical in all the varieties in spite of some differences in their nitrogen content. The true prolamin fraction (fraction 11) constitutes the largest percentage (40%) of total nitrogen for all the millets. The amino acid composition of the five protein fractions is independent of genotype.

Seed protein constitutes a major source of protein for food in Nigeria where millet is consumed to a large extent by adults and infants in the form of a dough called fura (Okoh and Eka, 1978).

Loosli (1974) showed that 70-90% of the 59 g of protein that are available per capita each day in Nigeria is from plant sources. These proteins have lower biological value than those originating from animal sources (eggs, milk, and meat) because they contain less of the essential amino acids

needed for growth and maintenance. Howe et al. (1965) indicated that for a consumption of cereal satisfying caloric requirements, the ingested protein would be quantitatively adequate if its quality was comparable to that of animal protein. The poor biological value of cereals cultivated in the subtropical and tropical regions, namely, pearl millet, corn, and sorghum, is due to their deficiency in lysine and tryptophan (Nwasike et al., 1979). Jambunathan and Mertz (1973) have shown from their work on sorghum that in order to improve the quality of cereal grains, it is desirable to know the distribution and amino acid composition of protein fractions isolated by selective extraction. Some studies on protein of pearl millet (Swaminathan et

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Table I. Protein Content and 1000 Grain Weight of Early and Late Maturing Millets

cultivars	protein ^a	1000 grain weight, g
early millets		
ex. Borno	13.7	8.3
ex. Benue	13.4	9.2
Nigerian composite	13.1	8.5
world composite	13.1	8.5
late millets		
ex. Riyom	16.3	10.8
ex. Tukururu	14.1	10.8
ex. Gashua	16.1	10.3

^a Grams per 100 g of dry matter.

al., 1970; Nwasike et al., 1979) have revealed that the poor biological value of this cereal is due to the high level of prolamins, which is low in lysine.

The most widely cultivated millets correspond to the photoinensitive or sensitive types that are early (EM) and later (LM) maturing, respectively. Preliminary analyses in our laboratory have shown that the first type has a lower protein content than the second one. Fractionation and characterization of the proteins from both types were performed with the aim to determine how variations in the nitrogen content modifies the distribution of protein fractions.

MATERIALS AND METHODS

Seven varieties of millet consisting of the four EM and three LM types were planted in June 1981 at the Institute for Agricultural Research Farm, Samaru. The EM and LM types matured in 90 and 120 days, respectively, but were harvested 30 days later to allow the plant to completely dry up. The 1000 grain weight was calculated from the mean weight of three batches of 100 grains that were randomly selected from bulked sample.

Whole seeds ground in a Thomas-Wiley laboratory mill, and the flour was defatted with petroleum ether (boiling point 40–60 °C). Fractionation of the seed protein was carried out on the defatted samples according to the Landry and Moureaux method (1970). Nitrogen in ground samples and various fractions was determined by the microkjeldahl method. The crude protein content was calculated by multiplying nitrogen value by 6.25. For each of the five fractions, an aliquot of the extract containing about 2.5 mg of nitrogen was hydrolyzed in a final volume of 100 mL of 6 N HCl for 24 h under reflux. Amino acid analysis was performed on a Technicon TSM-1 amino acid analyzer equipped with an integrator and printer. No other conditions were used for eliminating the effects due to incomplete hydrolysis.

RESULTS AND DISCUSSION

The protein contents and the 1000 grain weight of the EM millets appear to be lower than those of the LM ones (Table I). These differences are in agreement with the observation of Okonkwo (1983), who has shown from the

analysis of many varieties that grains of the EM millets were less dense than those of the LM types. Grains of LM millets therefore have the unique advantage of accumulating more protein in conjunction with more dry matter.

Table II gives the percentage distribution of nitrogen in the five Landry-Moureaux fractions for seven varieties. A very high extraction efficiency (99.5–101% of total nitrogen) was found. A similar value (100.7%) has been mentioned by Nwasike et al. (1979). Extraction yields as high as 95% have been seen for Italian millet (*Setaria italica*) by Monterio et al. (1982) while lower values have been observed with finger millets (*Eleusine coracana*) by Virupaksha et al. (1975) and Ramachandra et al. (1978).

Although the total seed protein was higher in LM millets compared with the EM ones (Table I), the distribution of proteins in the five solubility fractions was essentially similar irrespective of the varieties. Therefore, the true prolamins constitute the major fraction comprising 40% of the total nitrogen, and no appreciable increase in this percentage was seen with highest protein contents.

The albumin-globulin fraction accounts for about 22% of the protein in all the varieties tested, while the glutelin fraction accounts for about 21%. The prolamins-like proteins constitute the minor fractions (6% and 9%, respectively). Nwasike (1977) reported a prolamins content of 29–44% for five pear millet varieties with protein content ranging from 10.9% to 16.9%. However, the present results suggest that protein and prolamins contents are not highly correlated.

In contrast, a high positive correlation between these two parameters has been reported for Italian (Monteiro et al., 1982) and finger (Virupaksha et al., 1975) millets. Such differences suggest a great variability existing in the distribution of protein fractions among the millet species. As a matter of fact, the major fraction is represented by prolamins (over 40% of total nitrogen) in pearl and Italian millets and by prolamins-like proteins in finger millets, these showing this unique property with sorghum (Nwasike et al., 1979). However, finger millets resemble pearl millets from Nigeria for the similar level of true glutelin (about 21% of total nitrogen) whereas Italian millets are low in this fraction (less than 5%).

The amino acid compositions of the five protein fractions are given in Tables III and IV for EM and LM types. The results are averaged since only small differences in composition were recorded between homologous proteins originating from different varieties. It is also noteworthy that in the absence of specific assays for sulfur amino acids, differences in methionine content cannot be discussed. On the other hand, the low recovery of amino acid in fraction III could be due to extensive oxidative deamination during acid hydrolysis, as previously suggested (Nwasike et al., 1979). This is indicated by the high free ammonia value 11.2% in average).

The albumin-globulin fraction is the richest one in basic amino acids and the slight variations of composition recorded between EM and LM millets could be related to

Table II. Nitrogen Distribution in the Landry-Moureaux Fractions of Early and Late Season Millets^a

protein fractions	early millets				late millets		
	ex. Borno	ex. Benue	Nigerian composite	world composite	ex. Riyom	ex. Tukururu	ex. Gashua
I (albumin-globulin)	22.0	22.7	22.3	22.1	22.8	22.9	22.8
II (true prolamins)	40.1	40.1	40.0	40.0	40.6	40.5	40.7
III (prolamins-like)	6.4	6.8	6.1	6.0	6.1	6.4	6.4
IV (glutelin-like)	9.5	9.7	9.2	9.0	9.8	9.9	10.0
V (true glutelin)	21.3	21.1	21.1	21.1	21.5	21.1	21.4
total N extracted	99.5	100.6	98.9	98.5	101.1	100.9	101.4

^a Percent of total seed nitrogen.

Table III. Amino Acid Composition of Protein Fractions of Early Maturing Millets^a

	fractions				
	I	II	III	IV	V
Lys	6.1	0.6	tr ^b	2.0	3.2
His	5.3	1.4	0.6	3.1	3.8
Arg	14.2	0.8	0.4	2.4	6.3
Asp	15.0	7.0	2.5	2.4	7.8
Thr	4.2	2.9	1.7	3.0	3.7
Ser	5.5	4.9	2.0	2.2	3.7
Glu	18.5	23.6	9.6	18.9	13.2
Pro	2.9	7.9	3.5	6.3	7.5
Gly	5.2	0.7	1.0	3.1	3.2
Ala	6.9	8.9	4.7	3.6	5.2
Val	4.0	4.6	2.7	2.5	4.9
Met	0.1	0.5	0.1	0.6	1.0
Ile	3.9	5.3	3.2	2.3	4.4
Leu	5.4	13.7	5.3	4.4	6.3
Tyr	4.8	2.6	0.7	4.3	4.6
Phe	5.0	7.9	3.4	1.5	6.1
NH ₃	3.6	4.5	10.7	3.7	2.0
aa recovd, %	107.1	93.3	41.4	62.6	84.9

^a Grams of amino acid/100 g of protein; values are means from two varieties. ^b Trace.

Table IV. Amino Acid Composition of Protein Fractions of Late Maturing Millets^a

	fractions				
	I	II	III	IV	V
Lys	5.7	0.5	tr ^b	1.9	3.0
His	5.8	1.7	1.0	3.6	4.0
Arg	13.9	0.9	0.5	2.7	6.8
Asp	14.8	6.9	2.2	2.8	7.3
Thr	4.3	3.2	2.1	3.3	3.8
Ser	6.3	5.5	1.7	2.4	3.6
Glu	20.9	24.1	9.8	20.2	12.7
Pro	3.3	8.4	3.9	6.8	8.6
Gly	5.5	0.8	0.8	3.1	2.7
Ala	7.3	9.1	4.1	3.8	5.1
Val	3.9	4.3	2.5	2.4	5.1
Met	0.4	0.7	0.1	1.3	1.6
Ile	3.6	5.1	2.9	2.6	4.6
Leu	5.5	13.8	5.3	4.3	6.7
Tyr	4.9	2.8	0.7	4.8	4.3
Phe	5.1	8.5	3.6	1.8	6.3
NH ₃	3.0	4.5	11.8	3.4	2.3
aa recovd, %	111.2	95.6	41.2	67.8	86.2

^a Grams of amino acid/100 g of protein; values are means from three varieties. ^b Trace.

the presence of nonprotein nitrogen. Higher amounts of basic amino acids have been reported for the albumin-globulin fraction of Italian millets (Monteiro et al., 1982). The glutelin fraction is next to fraction I in having high contents of basic amino acids. The true prolamin com-

pared to other proteins is very poor in basic amino acids and rich in glutamic acid, alanine, phenylalanine, and leucine. The leucine:isoleucine ratio of about 3:1 in this fraction may further reduce its nutritional quality. In Italian millets, the prolamins have remarkably higher contents of glutamic acid than those reported here.

The second limiting amino acid in pearl millet is tryptophan. Although this amino acid was not assayed here, it is known from another work (Nwasike et al., 1979) that fractions I and V contain the most of the tryptophan-rich proteins. Compared to these fractions, fraction II has a lower level of tryptophan. From these data and those of Tables III and IV, it is concluded that improvement of the nutritional quality of pearl millet could be achieved by selecting varieties with grain poor in prolamin and rich in albumin-globulin and/or glutelin fractions. However, the absence of variability in the true prolamin percentage for cultivated millets suggests that the number of genotypes that could offer the genetic base for achieving prolamin reduction is limited.

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LITERATURE CITED

- Howe, E. E.; Jansen, G. R.; Gilfillan, E. W. *Am. J. Clin. Nutr.* **1965**, *16*, 315-320.
- Jambunathan, R.; Mertz, E. T. *J. Agric. Food Chem.* **1973**, *21*, 692-696.
- Landry, J.; Moureaux, T. *Bull. Soc. Chim. Biol.* **1970**, *52*, 1021-1036.
- Loosli, J. K. *BioScience* **1974**, *24*, 26-30.
- Monterio, P. V.; Virupaksha, T. K.; Rao, D. R. *J. Sci. Food Agric.* **1982**, *33*, 1072-1079.
- Nwasike, C. C. M.Sc. Dissertation, Purdue University, West Lafayette, IN, 1977.
- Nwasike, C. C.; Mertz, E. T.; Pickett, R. C.; Glover, U. D.; Chibber, B. A. K.; Van Scoyoc, S. W. *J. Agric. Food Chem.* **1979**, *27*, 1329-1331.
- Okoh, P. N.; Eka, O. U. *Savanna* **1978**, *7*, 67-70.
- Okonkwo, N. J. M.Sc. Dissertation, Ahmadu Bello University, Zaria, Nigeria, 1983.
- Ramachandra, G.; Virupaksha, T. K.; Shadaksharaswamy, M. *Phytochemistry* **1978**, *17*, 2487-2490.
- Swaminathan, M. S.; Naik, M. S.; Kaul, A. K.; Austin, A. "Proceedings, Symposium on the Improvement of Plant Protein by Nuclear Technique"; International Atomic Energy Agency: Vienna, Austria, 1970; p 165.
- Virupaksha, T. K.; Ramachandra, C.; Nagaraju, D. *J. Sci. Food Agric.* **1975**, *26*, 1237-1246.

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