Ramie—A High Protein Forage Crop for Tropical Areas

ROBERT L. SQUIBB

Instituto Agropecuario Nacional,
Guatemala

José Méndez, Miguel A. Guzmàn and Nevin S. Scrimshaw

Instituto de Nutrición de Centro América y Panamá

Reprinted without change of pagination from the Journal of the British Grassland Society. Vol. 9. No. 4. December, 1954.

RAMIE—A HIGH PROTEIN FORAGE CROP FOR TROPICAL AREAS

ROBERT L. SQUIBB
*Instituto Agropecuario Nacional, Guatemala**

José Méndez, Miguel A. Guzmàn and Nevin S. Scrimshaw
Instituto de Nutrición de Centro América y Panamá†

Ramie (*Boehmeria nivea*), known previously only as a fibre plant, shows promise of becoming an important new high-protein forage crop for livestock in tropical areas. Although ramie was introduced into Guatemala in early 1930, the plantings were soon abandoned, due to the difficulty of removing the fibre economically. The hardiness and adaptability of this genus to these areas is apparent, as plants from the initial introductions may be still found growing on a number of Guatemalan farms.

An early report from the INSTITUTO AGROPECUARIO NACIONAL (13) indicated that immature ramie plants contained approximately 18 per cent protein. SQUIBB *et al.* (20, 21, 22), using laboratory rats, baby chicks and adult hens, studied the nutritional value of a dehydrated ramie meal prepared from whole plants cut at approximately 20 inches in height. Five per cent ramie added to the diet supplied adequate quantities of vitamin A activity and riboflavin for both rats and chicken. Ramie also was found to be an excellent source of protein when included at a 20 per cent level in simplified all-vegetable protein rations for baby chicks.

The present report summarizes the relation between the height of cutting and the chemical content of fourteen nutrients in ramie forage. A dehydrated ramie meal was also assayed at minimum levels to observe further its effect on the growth, mortality, feed efficiency and the blood serum levels of vitamin A and total carotenoids of baby chicks. Preliminary observations and notes on the planting and on the management of ramie pastures for livestock are presented.

EXPERIMENTAL DESIGN

Relation of height of plant to the chemical content

In order to minimize possible effects of soil fertility and between-plant variation on the fourteen plant nutrients studied, the ramie was grown in a special bed 6 feet by 50 feet.‡ One hundred pounds of dry chicken manure was added to this area and all the soil within the bed was thoroughly mixed to a depth of 14 inches. Three hundred pieces of ramie root, originating from a single plant and cut to 3 inches in length, were sown at uniform intervals over the bed.

During the preliminary growth period while the stand was being established, the ramie

---

* *Instituto Agropecuario Nacional* (*IAN*), *Guatemala*, a technical agricultural organization operated jointly by the Governments of Guatemala and the United States of America.

† *Instituto de Nutrición de Centro América y Panamá* (*INCAP*), a human nutrition Institute supported by the Governments of Central America and Panama and administered by the Pan-American Sanitary Bureau, Regional Office of the World Health Organization.

‡ Located in Guatemala City, Guatemala, Central America, altitude 5,000 feet.

plants were cut five times at 35-day intervals. At the end of this preliminary period the ramie was well established. Before starting the experimental period, the plants of the entire bed were cut to approximately half an inch above the crown. A minimum amount of overhead irrigation was supplied during the two different collection periods.*

Two series of forage samples, 5, 10, 15, 20, 25, 30 and 35 inches in height (beginning of flower stage), were obtained at two different periods from this bed. Fifty to 150 individual stems, including tops and leaves, were harvested at random over the entire bed for each of the heights studied. Each group of forage samples of the same height was dried in an oven with warm moving air at a temperature not exceeding 42° C. The dried samples were then ground finely in a Wiley mill, sealed in all-glass containers and stored at −10° C. until the entire collection, including both series of cuttings, was ready for chemical analysis.

Moisture, crude fibre, ether extract, ash, calcium, silica and sand were determined by official A.O.A.C. methods (1). The digestion for nitrogen determination was done by the official method (1) and the distillation and titration as recommended by HAMILTON and SIMPSON (8). Iron was determined by the alpha, alpha′-dipyridyl method as described by HAHN (7). Riboflavin was determined by the fluorometric method of HODSON and NORRIS (10), thiamine according to HENNESSEY and CERECEDO (9) and ascorbic acid by the method of ROE and OESTERLING (18) as modified by BOLIN and BOOK (3). Phosphorus was determined by the procedure of LOWRY and LOPEZ (14) and the carotenoids by the chromatographic method of WALL and KELLEY (23). Two to four analyses were made on each sample for the above plant constituents. Microbiological assays were performed in duplicate. The methods of HORN *et al.* (11, 12) were used for the determination of lysine and methionine. Tryptophane was determined by the method of WOOLEY and SEBRELL (24) modified by autoclaving the material at 15 lb. for 30 minutes before enzymatic hydrolysis and by preparing the culture media according to the directions of GREENE and BLACK (6).

Vitamin A activity of dehydrated ramie meal

The vitamin A activity of ramie was determined in two experiments with chicks by including small quantities of the dehydrated meal in low-vitamin A rations. Three-day-old New Hampshire chicks of both sexes were used in the experiments. Each group of birds was housed in an all-wire cage equipped with a thermostatically controlled heater. The chicks received water and feed *ad-libitum* and were weighed individually every week. The low vitamin A basal ration fed in both experiments consisted of grm. (per 100 grm. ration): 30, sesame oil meal; 15, corozo oil meal; 4, cottonseed oil meal; 43·3, white corn; 3, minerals including the minor elements (21, 22); and 0·7, aureomycin-vitamin B_{12} feeding supplement.† To each 100 grm. of this basal diet were added: 0·02 grm. vitamin D supplement,‡ 0·20 mgrm. thiamine; 0·35 mgrm. riboflavin; 1·20 mgrm. calcium pantothenate; 1·50 mgrm. nicotinic acid; 0·35 mgrm. pyridoxine; and 125·00 mgrm. choline. Under the conditions of this laboratory this ration results in 100 per cent mortality

* Experiments were conducted during the dry season (January to May 1953). Ramie planted during this period at this altitude begins to flower at approximately 35 to 40 inches in height. During the wet season (May to October) the same plants will begin to flower at 40 to 65 inches in height.

† Aureofac, furnished by Dr. T. H. Jukes and the Lederle Laboratories Inc.

‡ Delsterol, vitamin D_3, 2,000 I.U. per grm., prepared by Dupont Company.

from avitaminosis A in less than 42 days. Ramie harvested at 20 inches in height was used in the preparation of the dehydrated meal for the assays.

In Experiment 1, 60 baby chicks were placed on the low-vitamin A ration for a period of 28 days. At the end of this period the 42 chicks, still alive, displayed typical symptoms of avitaminosis A. Thirty-six chicks were selected at random from these and distributed among four experimental groups of nine birds per group. These groups were fed the basal ration plus 0·0, 0·1, 0·3 and 0·6 per cent ramie meal during a 7-day curative period. At the end of this period each chick was bled by heart puncture and the blood sera analyzed for vitamin A and total carotenoids by the method of BESSEY *et al.* (2).

In Experiment 2, which covered a 5-week period, 192 chicks were distributed by weight among four experimental groups, 48 chicks per group. This assay differed from that of Experiment 1 in that all birds received the basal ration plus 0·0, 0·1, 0·4 and 0·8 per cent dehydrated ramie meal from the start of the feeding trial. At the end of the 5-week feeding period each chick was bled by heart puncture and the blood sera analyzed for vitamin A and total carotenoids.

RESULTS

Relation of height of plant to the chemical content

The results of the chemical analyses of the ramie forage samples are presented in Table 1. While the data for nitrogen are fairly uniform, there is slight to high variation between the two series of cuttings for the other thirteen nutrients studied. Crude fibre increased from 9·0 to 21·8 per cent with the increase in the height of the plant, while total nitrogen decreased uniformly and rapidly from 4·50 to 2·25 per cent. Ash decreased from 17·5 to 13·6 per cent and phosphorus

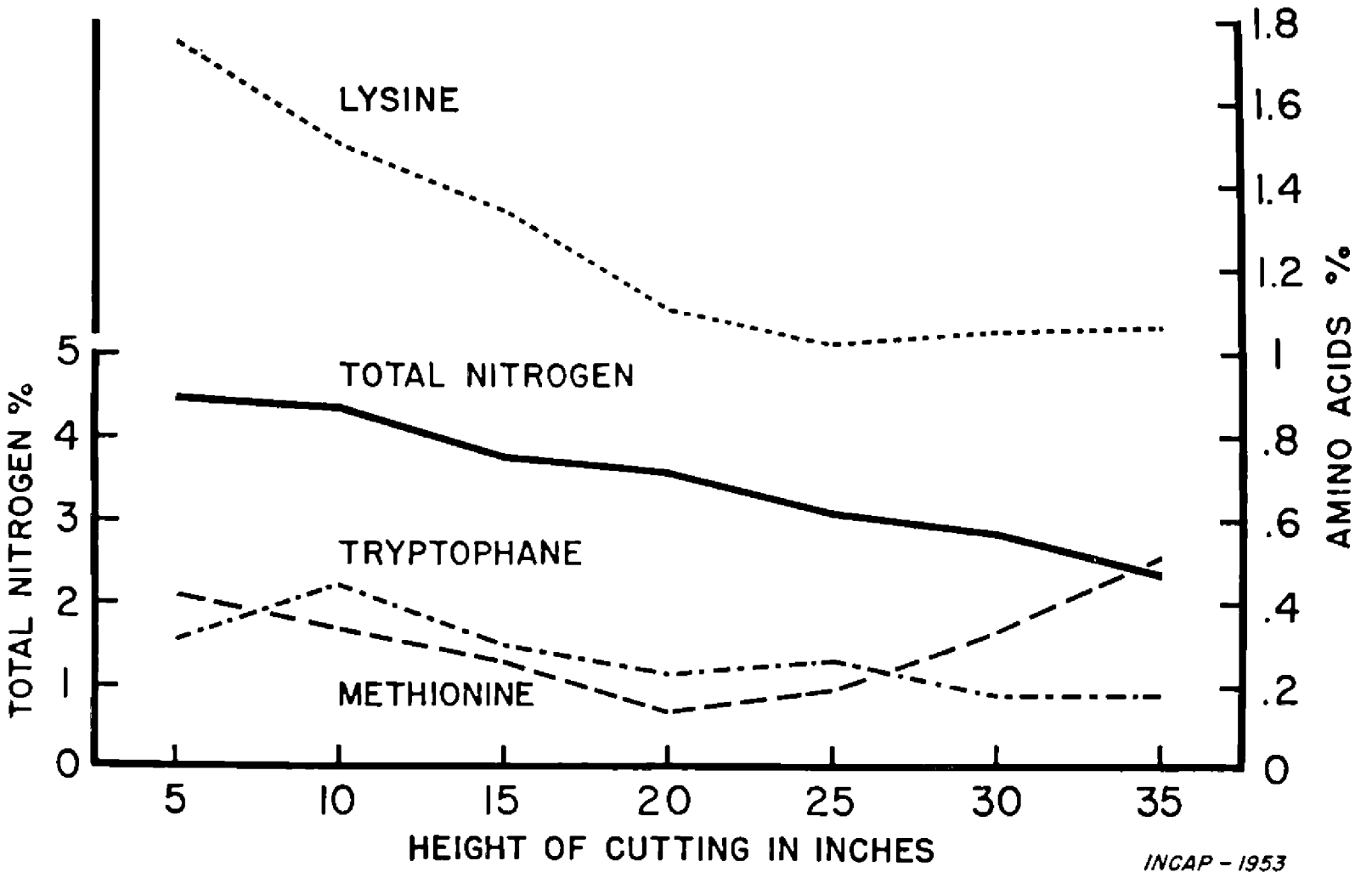

Fig. 1. Relation of the height of plant to the nitrogen, lysine, tryptophane and methionine content of the forage (10 per cent moisture)

TABLE 1. Relation of the height of the ramie plant to its nutrient content*

Plant height	Cutting	Nitrogen	Ether extract	Crude fibre	Carotenoids	Thiamine	Riboflavin	Ascorbic acid	Ash	Calcium	Phosphorus	Silica dioxide	Iron	Sand
inches		%	%	%	mgrm/100grm	mgrm/100grm	mgrm/100grm	mgrm/100grm	%	mgrm/100grm	mgrm/100grm	%	mgrm 100grm	%
5	1	4·48	3·6	10·5	13·81	0·32	1·63	135	16·9	3314	566	2·8	104	0·19
	2	4·50	3·7	9·0	11·72	0·37	1·50	—	17·5	4471	414		81	
10	1	4·34	3·9	11·0	13·99	0·41	1·82	140	15·5	3530	534	2·6	52	0·13
	2	4·33	4·2	12·7	—	0·13	1·82	—	17·4	4339	344		35	
15	1	3·73	2·8	13·8	13·45	0·39	1·36	50	14·7	3858	341	2·3	33	0·13
	2	3·77	4·2	13·3	12·20	0·18	1·19	104	16·7	4237	296		39	
20	1	3·54	2·6	10·9	13·23	0·32	1·56	51	14·7	3634	254	2·6	29	0·16
	2	3·61	3·9	14·0	12·02	0·18	1·21	53	15·3	4050	248		25	
25	1	3·14	2·8	14·4	9·22	0·38	1·40	52	14·8	2886	222		20	
	2	3·08	3·8	16·7	10·64	0·30	1·15	78	14·2	3924	215		25	
30	1	2·83	2·9	17·1	11·11	0·36	1·44	65	14·1	3321	189		22	
	2	2·88	3·6	15·9	9·80	0·25	1·12	37	13·7	4140	202		25	
35†	1	2·25	5·5	19·8	12·44	0·33	1·41	32	15·0	3722	170	3·0	31	0·19
	2	2·77	4·9	21·8	9·61	0·25	1·24	27	13·6	4048	234		33	

* Data are based on 10 per cent moisture.
† Start of flower stage.

from 566 to 170 mgrm./100 grm. The ascorbic acid values were reduced irregularly from 140 to 27 mgrm./100 grm. Iron was reduced from 104 to 20 mgrm./100 grm. at 25 inches in height, following which a slight average increase was observed. Carotenoids were reduced from a high of 13·81 to a low of 9·22 mgrm./100 grm. for plants 25 inches in height, beyond which a slight average increase was observed. With little or no apparent relation to the height of the plant, riboflavin ranged from 1·63 to 1·12 mgrm./100 grm., thiamine from 0·13 to 0·41 mgrm./100 grm., calcium 2886 to 4471 mgrm./100 grm., silica 2·3 to 3·0 per cent; sand, 0·13 to 0·19 per cent, and ether extract from 2·3 to 6·1 per cent. As may be seen in Fig. 1, lysine decreased from 1·75 to 1·05 per cent at 25 inches in height and then maintained approximately this level. Tryptophane ranged from 0·31 to 0·18 per cent, decreasing only slightly with increasing height of plant. Methionine, on the other hand, decreased from 0·41 to 0·14 per cent at 20 inches in height of the plant and then increased from 0·14 to 0·57 per cent at the beginning of the flowering stage.

Vitamin A activity of ramie meal

The mortality and serum levels of vitamin A and carotenoids of baby chicks depleted of their vitamin A reserves during a 4-week period and then supplemented with dehydrated ramie meal for a 7-day curative period are presented in Table 2. These data show that 0·1 to 0·6 per cent ramie meal prevented mortality during the 7-day period and increased serum vitamin A and total carotenoids. When ramie meal was included in the ration during a 5-week feeding trial, Experiment 2, the data observed in Table 3 confirmed those of Experiment 1. In Table 3,

TABLE 2. Experiment 1. Mortality and serum vitamin A and carotenoid levels of depleted chicks fed various percentages of ramie for a 7-day curative period

Ramie	Number of chicks	Mortality	Serum	
			Vitamin A	Carotenoids
%		%	mcg/100 cc.	mcg/100 cc.
0·0	9	33	0	19
0·1	9	0	3	42
0·3	9	0	10	90
0·6	9	0	23	206

evidence is presented that as the percentage of ramie meal was increased from 0·1 to 0·8 per cent, mortality was reduced and increases were observed in weight gain, efficiency of feed utilization and serum levels of vitamin A and carotenoids of the baby chicks.

TABLE 3. Experiment 2. Weight gains, mortality and serum vitamin A and carotenoid levels of chicks fed various percentages of ramie over a 35-day feeding period

Ramie	Number of chicks	Final weight	Mortality	Feed efficiency*	Intake carotenoids	Serum	
						Vitamin A	Carotenoids
%		grm.	%		mgrm.	mcg/100 cc.	mcg/100 cc.
0·0	48	—	77	—	—	—	—
0·1	48	220	14	4·35	0·13	7	47
0·4	48	245	6	4·33	0·55	13	125
0·8	48	272	0	4·16	1·19	20	225

* Grm. of feed to produce 1 grm. gain in weight

Discussion

The chemical analyses and the baby chick vitamin A activity assays presented in this report show ramie to be a highly nutritious forage plant when utilized in its immature stages. A comparison of the chemical data on cuttings shows considerable variation between the two series for several of the nutrients analysed. Variations in sample preparation and in the results of the analytical methods can account for only a small part of this variability. The excellent agreement between the corresponding values for nitrogen in the two series may be considered an indication of the uniformity of the sampling technique employed. It is apparent that height of cutting has an important effect on nutrient content, although for certain plant constituents there is also considerable variation between samples cut at the same height.

Although the mature plant has always been considered a source of one of the strongest and best of the vegetable fibres, the young growing ramie plant has less fibre and more protein than alfalfa harvested before bloom stage. Since ramie can be grown from sea level to 6,000 feet altitudes throughout the tropics, while alfalfa may be grown only in restricted areas, its advantages are considerable. Ramie forage grown in other than the Guatemalan area is also high in protein. Data obtained by the Tingo Maria Agricultural Station, Peru, South America (19) show ramie forage samples from that locality to have similar amounts of protein to comparable samples harvested in Guatemala, Central America. Work at the University of Florida Agriculture Experiment Station (4) has shown that the tops and leaves of the mature ramie plant, by-products left after the stems were stripped of their fibre, to be high in protein.

The relation of tryptophane, methionine and lysine to the height of the ramie plant, as observed in the studies reported here, resemble those reported by Reber and MacVicar (17) for several cereal grasses. These authors showed that tryptophane remained fairly constant while lysine and methionine decreased during the early growth period. Both lysine and methionine then increased as the plants entered the early heading stage, while tryptophane remained constant.

It is apparent that the ramie plant absorbs considerable quantities of minerals from the soil. The high ash content of the various forage samples studied was similar to that of Florida samples consisting of the tops and leaves of the mature plant (15). While complete data are not available on the silica and sand content of all the ramie forage samples, it is evident from Table 1 that these two plant constituents are not present in excessive quantities. The high absorption of minerals by the ramie plant may be detrimental for livestock raised in areas having large quantities of molybdenum in the soils, as toxic quantities of this element may be absorbed. Molybdenum toxicity may be countered in part by the addition of small quantities of copper in the ration (5).

The carotenoid content of ramie compares favourably with that of high-quality alfalfa meals. The baby chick assays showed these carotenoids to have a high vitamin A activity. Dehydrated ramie meal fed at 0·6 to 0·8 per cent of the baby chick rations was found to maintain serum vitamin A and carotenoid levels which were considered adequate when compared to unpublished data of the authors. High vitamin A activity of ramie meal is important to the areas of the American tropics having prolonged dry seasons, where many last as long as six months. Small quantities of a dehydrated ramie meal added to the diet during dry seasons could supply all of the vitamin A required by most animals.

Notes on the cultivation and management of ramie pastures

Considerable research will be required before sufficient data are available on the cultivation and management of ramie pastures for livestock. In general, ramie forage is highly palatable,

although some animals will not eat it readily the first time it is offered. Swine, sheep, cattle and horses have grazed on ramie pastures at the tropical sub-station Chocolá of the IAN. Thus far no cases of bloat have been observed.

While ramie grows very rapidly, it does so only after being well established. This may take from 60 to 190 days, depending on the altitude, soils and moisture available. Ramie planted in the Guatemalan lowlands has been successfully pastured or cut as a silage crop at 15 to 25-day intervals over a 2-year period. Small forage test plots cut at 20 inches in height at the IAN in Guatemala City have averaged more than 30 to 45 tons of green forage per acre per year. While the data are not complete, it appears that these yields will be greatly increased in Guatemalan lowland areas.

Several procedures have been used to establish ramie pastures in Guatemala. Small pieces of root 3 inches in length can be sown 2 inches deep at 18 to 36-inch intervals. Mature stems also may be rooted in beds when embedded at an angle in moist, well-drained soils. The rooted plants are then transplanted into the pastures. Ramie seed has been germinated by mixing it with sand and sprinkling the mixture on well prepared moist soil in small greenhouse boxes. These are kept in glass greenhouses until the seedlings are ready for transplanting.

Weed growth has been successfully held down by cultivation and the interplanting of new ramie pastures with pigeon peas (*Cajanus indicus*) and cowpeas. At present, procumbrant species of the genus *Desmodium* are showing promise as permanent mixtures in ramie pastures (Fig. 2). It is possible that interplanting ramie pastures with legumes will not only aid in controlling weeds but also aid in reducing the loss of nitrogen from the soil. Ramie pastures which have been over-grazed and heavily invaded with weeds have been successfully rejuvenated by discing the fields. In addition to controlling the weeds, this tends to chop up and redistribute the ramie roots.

While information may be obtained from the report of NELLER (16) which discussed the fertilizer requirements and fibre yields of ramie plantings in the Florida Everglades, little is known of the fertilization of ramie pastures. In Guatemala, chicken and cow manures are the only fertilizers which have been used to date. Due to the high protein content of the plant, it may be necessary to add nitrogen to the soil. This would be especially so if the pastures were used to supply silage crops.

SUMMARY

Results of chemical analyses of ramie forage samples cut at 5, 10, 15, 20, 25, 30 and 35 inches in height are presented. These analyses show total nitrogen decreases with increasing height of cutting from 4·50 to 2·25 per cent; ash from 17·5 to 13·6 per cent; and phosphorus from 566 to 170 mgrm./100 grm. Ascorbic acid values were reduced irregularly from 140 to 27 mgrm./100 grm. Iron was reduced from 104 mgrm./100 grm. at 5 inches to 20 mgrm./100 grm. at 25 inches in height, following which the values remained fairly constant. Carotenoids similarly were reduced from 13·81 mgrm./100 grm. at 5 inches to 9·22 mgrm./100 grm. at 25 inches, following which they increased slightly. With little or no apparent relation to the height of the plant, riboflavin ranged from 1·63 to 1·12 mgrm./100 grm.; thiamine, from 0·13 to 0·41 mgrm./100 grm.; calcium, from 2886 to 4471 mgrm./100 grm.; silica, 2·3 to 3·0 per cent; sand, 0·13 to 0·19 per cent; and ether extract, from 2·3 to 6·1 per cent. Crude fibre increased from 9·0 to 21·8 per cent as the height of the plant increased.

Fig. 2. Trial plots of Ramie and *Desmodium intortum*

Lysine decreased from 1·75 to 1·05 per cent at 25 inches in height, then maintained approximately this level. Tryptophane decreased from 0·31 to 0·18 per cent with the increased height of the plant. Methionine decreased from 0·41 to 0·14 per cent at 20 inches in height of the plant and then increased to 0·57 per cent at the beginning of the flowering stage.

Assays with baby chicks showed that 0·6 to 0·8 per cent of dehydrated ramie meal prevented symptoms of avitaminosis A and maintained adequate serum levels of vitamin A and carotenoids in baby chicks.

Although the need for additional research on the agronomic aspects of the cultivation of ramie as a forage crop is stressed, ramie is proposed as an important new high-protein forage crop for the animal industry of tropical and sub-tropical areas. Ramie was found to be most nutritious when harvested or pastured at less than 20 inches in height, at which height it contains slightly more protein and less fibre than alfalfa before the bloom stage.

ACKNOWLEDGEMENTS

José Morales aided in the care and harvesting of the ramie plants and the feeding of the baby chicks. Mr. Antonio Fuentes made the silica and sand determinations, and Mr. Francisco Aguirre and Mr. Alvaro Aguirre carried out the amino acid determinations. Mrs. Carlotha de Funes, Miss M. L. Guzmán and Miss Eva Judith Colón gave valuable technical assistance in the serum vitamin assays.

REFERENCES

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1945. Official and Tentative Methods of Analysis of the Association of Agricultural Chemists. 6th Ed. Washington, D.C.
2. BESSEY, O. A., LOWRY, O. H., BROCK, M. J., and LOPEZ, J. A. 1946. The determination of vitamin A and carotene in small quantities of blood serum. *J. Biol. Chem.* Vol. 166. pp. 177–188.
3. BOLIN, D. W., and BOOK, L. 1947. Oxidation of ascorbic acid to dehydroascorbic acid. *Sci.* Vol. 106 p. 451.
4. DAVIS, G. K., MEHRHOF, N. R., COMAR, C. L., SINGER, L., and BONEY, K. 1947. Ramie meal a new feed. Annual Rept. Univ. Florida Agric. Exp. Sta.
5. ————————. 1950. Personal communication.
6. GREENE, R. D., and BLACK, A. 1944. The microbiological assay of tryptophane in proteins and foods. *J. Biol. Chem.* Vol. 155. pp. 1–8.
7. HAHN, P. F. 1945. Radioactive iron procedures. Purification electroplanting and analysis. *Ind. Eng. Chem. Anal. Ed.* Vol. 17. pp. 45–46.
8. HAMILTON, L. F., and SIMPSON, S. G. 1947. Talbot's Quantitative Chemical Analysis. 9th Ed. The McMillan Company, New York.
9. HENNESSEY, D. J., and CERECEDO, L. R. 1939. The determination of free and phosphorylated thiamine by a modified thiochrome assay. *J. Amer. Chem. Soc.* Vol. 61. pp. 179–183.
10. HODSON, A. Z., and NORRIS, L. C. 1939. A fluorometric method for determining the riboflavin content of foodstuffs. *J. Biol. Chem.* Vol. 131. pp. 621–630.
11. HORN, M. J., JONES, D. B., and BLUM, A. E. 1946. Microbiological determination of methionine in proteins and foods. *J. Biol. Chem.* Vol. 166. pp. 321–326.
12. ————————————————. 1947. Microbiological determination of lysine in proteins and foods. *J. Biol. Chem.* Vol. 169. pp. 71–76.
13. INSTITUTO AGROPECUARIO NACIONAL. 1948–49. Annual Report, Guatemala, C.A.
14. LOWRY, O. H., and LOPEZ, J. A. 1946. The determination of inorganic phosphate in the presence of labile phosphate esters. *J. Biol. Chem.* Vol. 162. pp. 421–428.
15. MEHRHOF, N. R., DAVIS, G. K., and DRIGGERS. 1950. Ramie meal in chick rations. Univ. Florida, Agric. Exp. Sta. Cir. S-20.
16. NELLER, J. R. 1945. Culture fertilizer requirements and fibre yields of ramie in the Florida Everglades. Univ. Florida Agric. Exp. Sta. Bull. 412.
17. REBER, E., and MACVICAR, R. 1953. The nitrogen composition of cereal grasses. III. Amino acid distribution in field clippings and growing plants. *Agron. J.* Vol. 45. pp. 17–21.
18. ROE, J. H., and OESTERLING, M. J. 1944. The determination of dehydroascorbic acid and ascorbic acid in plant tissues by the 2, 4-dinitrophenyl hydrazine method. *J. Biol. Chem.* Vol. 152. pp. 511–517.
19. SINCLAIR, L. R. 1953. Unpublished data.
20. SQUIBB, R. L., GUZMAN, M., and SCRIMSHAW, N. S. 1953. Carotene and riboflavin retention and serum vitamin levels in vitamin A depleted rats fed four forage meals, achiote and African palm oil. *Turrialba.* Vol. 3. pp. 91–94.

21. SQUIBB, R. L., GUZMAN, M., and SCRIMSHAW, N. S. 1953. Dehydrated *desmodium*, kikuyu grass, ramie and banana leaf forages as supplements of protein, riboflavin and carotenoids in chick rations. *Poultry Sci.* Vol. 32. pp. 1078–1083.

22. ———————————————. 1953. Effect of pasture, dehydrated ramie meal and sex on five blood constituents of New Hampshire chickens. *Poultry Sci.* ibid. pp. 953–957.

23. WALL, M. E., and KELLEY, E. G. 1943. Determination of pure carotene in plant tissue, rapid chromatographic method. *Ind. Eng. Chem. Anal. Ed.* Vol. 15. pp. 18–20.

24. WOOLEY, G. J., and SEBRELL, W. H. (JR.). 1945. Two microbiological methods for the determination of l (—)-tryptophane in proteins and other complex substances. *J. Biol. Chem.* Vol. 157. pp. 141–151.

(*Received* 12 *May*, 1954)

MADE AND PRINTED IN GREAT BRITAIN BY
CULVERLANDS PRESS LTD., WINCHESTER.