DEHYDRATED DESMODIUM, KIKUYU GRASS, RAMIE AND BANANA LEAF FORAGES AS SUPPLEMENTS OF PROTEIN, RIBOFLAVIN, AND CAROTENOIDS IN CHICK RATIONS

BY

R. L. SQUIBB, M. GUZMÁN AND N. S. SCRIMSHAW

# Dehydrated Desmodium, Kikuyu Grass, Ramie, and Banana Leaf Forages as Supplements of Protein, Riboflavin, and Carotenoids in Chick Rations

ROBERT L. SQUIBB, MIGUEL GUZMÁN AND NEVIN S. SCRIMSHAW<sup>1</sup>

(Received for publication April 6, 1953)

If A successful poultry industry is to be developed in countries of the American Tropics, local vegetable sources of protein, riboflavin, and vitamin A activity must be exploited. Forages grow rapidly in the Tropics; and several promise to contribute large quantities of nutrients essential for adequate poultry rations.

Desmodium, Desmodium intortum, has been shown to be equal to a United States alfalfa meal in baby chick rations (Squibb et al., 1950) and is found throughout the American Tropics as well as in the southern United States.

Kikuyu grass, Pennisetum clandestinum, grows well at most altitudes in Central America. It has been found to be an excellent supplement to corn and sesame oil meal for swine on dry lot (Squibb et al., 1951). As a pasture, according to unpublished data by Squibb, it also maintains high serum levels of riboflavin, vitamin A, and carotenoid levels in laying hens.

<sup>1</sup> Dr. Squibb is Animal Husbandman, Foreign Agricultural Service, U. S. Department of Agriculture, assigned to the Instituto Agropecuario Nacional, Guatemala, a technical agricultural service organization operated jointly by the Governments of Guatemala and the United States; cooperation of the latter is under the Point IV program in Guatemala, administered by the Technical Cooperation Administration, U. S. Department of State, through the Institute of Inter-American Affairs.

Mr. Guzmán and Dr. Scrimshaw are on the staff of Instituto de Nutrición, de Centro-América y Panamá, Guatemala, 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.

Another plant that grows well in these areas is ramie (Boehmeria nivea), an ancient fiber plant. A report from Florida (Davies et al., 1947) shows that the leaves and tops of the ramie plant may replace alfalfa meal in the baby chick diet and are excellent for cattle.

The banana plant also is an important feed in the Central American areas. Lewy van Severen and Carbonell (1949), using goats to determine digestion coefficients, have shown that a banana leaf meal is highly nutritious.

The studies here described were undertaken to determine the value of dehydrated desmodium, kikuyu grass, ramie, and banana leaf forage meals to supplement protein, riboflavin, and carotenoids in baby chick rations. The effect of these meals on blood serum levels of protein, ribofla ascorbic acid, carotenoids, and vitamin A also was observed.

## METHODS AND RESULTS

Three experiments were carried out in a room with uniform temperature. Three-day-old straight-run baby chicks from the Instituto Agropecuario Nacional's flocks of New Hampshires were distributed by weight among the experimental groups. The chicks were reared in all-wire cages with raised screen floors, each cage holding 12 chicks. Feed and water were provided ad libitum. All diets were mixed fresh weekly and stored in glass jars in a dark cabinet. Individual weights of the chicks were recorded weekly for a period of 5 weeks.

												_	
Forage meal	Mois- ture	Ether ex- tract	Crude fiber	Nitro- gen	Ash	Cal- cium	Phos- phorus	Iron	Carot- enoids	Thia- mine	Ribo- flavin	Nia- cin	Ascor- bic acid
Desmodium Kikuyu grass Banana leaves Ramie	% 3.0 7.3 3.3 3.9	gms./% 4.54 1.27 4.18 3.18	gms./% 20.7 19.0 18.6 11.9	gms./% 2.06 2.61 2.58 3.59	gms./% 7.4 9.7 12.1 17.7	mg./% 1,200 231 575 4,544	mg./% 269 188 211 136	mg./% 46 21 16 74	mg./% 17.9 23.8 15.5 12.8	mg./% 0.37 0.12 0.32	mg./% 0.75 1.89 4.56 0.71	mg./% 5.09 4.62 5.64 5.38	mg./% 18.0 24.9 26.9 26.9
Alfalfa:¹ Hay Meal	9.5 7.4	2.00 2.80	28.9 24.3	2.36 3.02	8.2 9.5	1,470 1,500	240 350	<u>25</u>	2.5 9.6	0.29 0.44	1.38 1.48	3.86 —	=

TABLE 1.—Chemical composition of forage meals used in Experiment 1 as compared with that of alfalfa hay and meal

Table 2.—Response of New Hampshire chicks in Guatemala, in both weight and feed efficiency, to various percentage levels of 3 forage meals in a simplified protein ration, Experiment 1

Forage meal, and response studied	0 percent	5 percent	10 percent	20 percent
Kikuyu grass: Average weight (grams) Feed efficiency (grams of feed to produce	193	209	235	238
1-gram gain in weight)	2.80	2.74	2.63	2.83
Ramie: Average weight (grams)	179	189	238	249
Feed efficiency (grams of feed to produce 1-gram gain in weight)	2.90	2.85	2.75	2.81
Banana leaves: Average weight (grams)	186	208	214	237
Feed efficiency (grams of feed to produce 1-gram gain in weight)	2.81	2.77	2.88	2.95

Note.—Figures are averages of 2 feeding tests, 24 chicks per group per test. Linear component of each trial was highly significant.

The forage meals tested were prepared especially for these experiments. The desmodium was cut when it was approximately 16 inches high and included stems and leaves; the kikuyu grass was cut at 5 inches; the ramie was cut at 16 inches and included the entire plant; the banana leaves were cut fresh, and the heavy center vein of the leaves was removed. These green forages were dehydrated in an oven with moving warm air at 115°F. and then ground. The dried meals were then chemically analyzed (see Table 1); data indicate that all are relatively high in essential nutrients, comparing favorably with alfalfa hay and alfalfa meal.

Experiment 1. In Experiment 1, kikuyu grass and ramie and banana leaf meals

were tested as supplements to a low-lysine simplified vegetable-protein basal diet in 3 duplicate trials. The basal diet consisted of the following: Sesame oil meal, 45; ground corn, 49.7; salt mixture, including trace elements, 3; Vita Rich,<sup>2</sup> 1.6; vitamin B<sub>12</sub>-antibiotic supplement,<sup>3</sup> 0.7. The forage meals were fed at 0-, 5-, 10-, and 20-percent levels, replacing part of the corn and the sesame oil meal in the basal ration.

<sup>&</sup>lt;sup>1</sup> Data for alfalfa are from F. B. Morrison's, Feeds and Feeding, 1936.

<sup>&</sup>lt;sup>2</sup> A vitamin concentrate, claimed by the manufacturer, Thompson-Hayward Chemical Co., to contain (in a carrier of sardine and whey solubles, fish liver and glandular meals etc.) per pound, not less than 300 mg. riboflavin, 250 mg. pantothenic acid, 10,000 mg. choline, 20 mg. thiamine, 300 mg. niacin, 0.5 mg. vitamin B<sub>12</sub>, 90,800 AOAC chick units of vitamin D, and 90,800 U.S.P. units of vitamin A.

<sup>&</sup>lt;sup>3</sup> Courtesy Dr. T. H. Jukes, Lederle Laboratories.

	Average	Food	Blood constituents						
Ration	final weight	Feed efficiency	Ribo- flavin	Total proteins	Ascorbic acid	Carote- noids	Vitamin A		
Without supplement With supplement of— Riboflavin Kikuyu Desmodium Ramie Banana leaves	gms. 289 404 361 392 345 367	gms. fed to produce 1-gm. gain 2.64 2.09 2.04 2.08 2.23 2.27	γ/% 0.39 0.94 0.91 1.10 0.85 0.74	mg./% 4.01 3.45 3.75 3.87 4.03 3.93	mg./% 1.38 1.66 1.89 2.23 2.10 1.90	γ/% 280 178 1,110 704 715 483	γ/% 44.7 45.1 67.9 57.0 55.4 58.2		
Least significant differ-									

0.33

0.20

TABLE 3.—Weight gain, feed efficiency, and level of 5 blood constituents in New Hampshire chicks in Guatemala fed a low-riboflavin ration without and with supplements of crystalline riboflavin and forage meals, Experiment 2

Note: Figures are averages for the 24 chicks in each group.

Data presented in Table 2 indicate that as the percentage of the forage meals was increased in the basal ration, an increase in the average weight of the chicks resulted (linear component was highly significant). These data indicate that kikuyu grass and ramie and banana leaf forages are useful supplements, supplying lysine and other essential nutrients which were not present in adequate quantities in the simplified basal rations.

ence at 5-percent level

Experiment 2. In Experiment 2, desmodium, kikuyu grass, and ramie and banana leaf forage meals were tested as sources of riboflavin in a low-riboflavin basal ration. The basal ration consisted of the following: Corozo oil meal, 30; sesame oil meal, 15; cottonseed oil meal, 15; vitamin test casein, 2.5; white corn, 33.5; salt mixture, including trace elements, 3; vitamin B<sub>12</sub>-antibiotic supplement, 0.7; cod liver oil (1,800 IU's of vitamin A and 400 IU's of vitamin D per gram), 0.3; and crystalline B complex vitamins (Squibb and Wyld, 1952) minus riboflavin.

The meals were fed at 5 percent of the basal ration, replacing 4.5 percent of the corn and 0.5 percent of the casein. Two

control groups were included in this experiment: one received the basal ration; the other, the basal ration plus crystalline riboflavin.

0.26

177

11.5

The chicks fed a low-riboflavin basal ration including crystalline riboflavin or 5 percent forage meals grew significantly faster than the control groups (see Table 3). The efficiencies of feed utilization were similar for the groups fed the forage meals and the group fed the basal diet plus crystalline riboflavin. The basal control group showed slightly less efficiency in feed consumption.

Experiment 3. In Experiment 3, desmodium, kikuyu grass, and ramie and banana leaf meals were assayed as sources of vitamin A activity in a basal ration low in vitamin A. The basal ration was the same as that used in experiment 2, except that crystalline riboflavin was included and that Delsterol, instead of cod liver oil, supplied the vitamin D. The weight of the cod liver oil was replaced by white corn. The forage meals were included at 5 percent of this ration replacing 4.5 percent of the corn and 0.5 percent of the casein. Two control groups were used—

(1) a negative control group, which re-

ceived the basal ration without vitamin A supplementation, and (2) a positive control group, which was fed the basal ration plus 105 IU's vitamin A per 100 grams of ration.

The group fed the low-vitamin-A basal ration suffered 100 percent mortality as the result of avitaminosis A. The addition of 105 IU's of vitamin A per 100 grams of the basal ration prevented mortalities from avitaminosis A although the added vitamin A was not enough to promote chick growth equal to that of the groups fed the basal ration with one of the forage meals. The chicks fed the forage meals grew significantly faster and had better efficiencies of feed utilization than did the group fed the basal ration with vitamin A added.

Blood samples. Blood samples were obtained by heart puncture from 10 chicks selected at random from each of the groups in Experiments 2 and 3. Serum of the samples from Experiment 2 was analyzed for riboflavin (method, Burch et al., 1948), total proteins (method, Lowry and Hunter, 1945), ascorbic acid (method, Lowry et al., 1945, and Goodland et al., 1949; was modified by using a solution of

copper sulfate and thiourea instead of norite), and carotenoids and vitamin A (method, Bessey et al., 1946). Serum from Experiment 3 was analyzed for carotenoids and vitamin A.

Analyses of the blood serum from chicks in Experiment 2 may be summarized as follows (for data, see Table 3):

- 1. Serum levels of *riboflavin* were significantly higher in the groups fed the forage meals and in the groups fed the control ration plus crystalline riboflavin than in the group fed the negative control ration. Of the four forages, desmodium meal maintained the highest level of serum riboflavin; banana leaf meal resulted in the lowest.
- 2. Total proteins were significantly lower in the groups fed the basal ration plus crystalline riboflavin and in the group fed the kikuyu grass. In the groups fed desmodium, ramie, and banana leaf forage meals, protein levels were similar to those of the negative control group.
- 3. Ascorbic acid was significantly higher in the groups fed the forage meals and slightly, but not significantly, higher in the group fed the basal ration supplemented with crystalline riboflavin.
- 4. Carotenoids were significantly higher in the groups fed the forage meals than in the groups fed the basal rations with and without crystalline riboflavin added. Among the forage meals kikuyu grass maintained the highest blood level of carotenoids; desmodium and ramie

TABLE 4.—Weight gain, feed efficiency, and blood-serum levels of carotenoids and vitamin A in New Hampshire chicks in Guatemala fed a low-vitamin-A basal ration without and with supplements of vitamin A and forage meals, Experiment 3

D. Alian	Average	Feed	Blood-serum level			
Ration	final weight	efficiency	Carotenoids	Vitamin A		
	gms.	gms. fed to pro- duce 1-gm. gain	γ/%	γ/%		
Without supplement With supplement of—	1,	duce 1-gm. gam	1	1		
Vitamin A	267	2.93	39	15		
Kikuyu grass	345	2.77	1,028	60		
Desmodium	340	2.80	670	51		
Ramie	334	2.84	1,115	52		
Banana leaves	361	2.88	660	50		
Least significant differ- ence at 5-percent level	31	_	351	11		

<sup>&</sup>lt;sup>1</sup> Mortality was 100 percent.

Note: Figures are averages for the 24 chicks in each group.

- gave a lower level. Banana leaf meal resulted in a significantly lower level than did any of the other forage meals.
- 5. Vitamin A was also highest in the group fed the kikuyu grass meal and similar in the groups receiving the other forage meals. All groups fed the forage meals had higher vitamin A serum levels than did the control groups, with or without additions of riboflavin.

Analysis of blood serum from chicks in Experiment 3 (see Table 4) showed that the serum levels of carotenoids and vitamin A were significantly higher in chicks fed the forage meals than in those fed the basal ration with or without additions of vitamin A.

#### DISCUSSION

The chemical data reported here indicate that forage meals of dehydrated desmodium, kikuyu grass, and ramie and banana leaf are equal or, in some nutrients, superior to alfalfa meals.

The results of Experiment 1 (Table 2) show that the best growth rate of chicks was obtained at the 20-percent level of the forage meals tested. These data were similar to those observed by Squibb et al. (1950), who found that 20 percent of a dehydrated desmodium meal produced the best growth when included in a simplified all-vegetable-protein ration containing sesame oil meal as the principal source of protein. However, further work (Squibb and Wyld, 1950) demonstrated that, when more than 10 percent of desmodium meal was included in rations containing a combination of three vegetable oil meals, the growth of the chicks was depressed. And unpublished data of the Instituto Agropecuario Nacional indicate that dehydrated kikuyu grass, ramie and banana leaf forage meals, when fed at more than 10 percent of rations containing several vegetable protein oil meals, depress chick growth. These meals therefore contain chick-growth-depressing factor(s) similar to those reported for desmodium meal (Squibb and Wyld, 1950) and alfalfa meals (Cooney et al., 1948).

It may be observed that the serum riboflavin levels (Table 3) of the various groups of chicks were not always related to the chemical content of the forage (Table 1). Banana leaf meal, for example, had the highest riboflavin content among the forages; nevertheless, it resulted in the lowest serum riboflavin level in the chicks. Desmodium on the other hand, which had the lowest riboflavin content, maintained the highest riboflavin level in the chicks. A probable explanation of these data might be the availability of the riboflavin; or it might be an effect of intestinal synthesis of this vitamin. On the other hand, the ascorbic acid and carotenoid blood levels of the chicks appeared to be related to the chemical content of the forages.

The data (Table 4) show that the forage meals studied, when fed at a 5 percent level in rations low in vitamin A, will prevent avitaminosis A and will maintain high serum levels of carotenoids and vitamin A. The lack of significance in the differences of the levels of carotenoids and vitamin A among the groups fed the four forage meals was due to a high withingroup variation for these nutrients.

It is evident that desmodium, kikuyu grass, ramie and banana leaf forages, properly harvested and dehydrated, are excellent sources of protein, riboflavin, and vitamin A activity. The blood data show that these meals are capable of maintaining in baby chicks adequate serum levels of riboflavin, ascorbic acid, carotenoids, vitamin A, and total proteins. These data are important to areas which are unable as yet to fortify poultry rations with commercial concentrates containing the essential nutrients studied. Care will need to be exercised in the harvesting and preparation of forages as it is reasonable to expect that considerable

variation of nutritive quality may occur within meals due to differences in time and height of cutting, in climate, and in the dehydration and storing of the finished product.

#### **SUMMARY**

The chemical content of dehydrated desmodium (Desmodium intortum), kikuyu grass (Pennisetum clandestinum), ramie (Boehmeria nivea), and banana leaf forage meals was found to be equal and, in some nutrients, superior to alfalfa meals.

Feeding trials with baby chicks demonstrated that these forage meals were excellent sources of proteins, riboflavin, and vitamin A activity. When fed at 5percent of basal rations low in vitamin A or riboflavin, the forage meals provided carotenoids and adequate for growth. Analyses of the blood of the baby chicks showed that the four forage meals also maintained high levels of serum riboflavin, ascorbic acid, vitamin A, and carotenoids. These data are important for those areas which are as yet unable to fortify poultry rations with vitamin concentrates containing these nutrients.

## ACKNOWLEDGMENTS

The financial aid of the Instituto de Fomento de la Producción, Guatemala, is gratefully acknowledged.

Chemical analyses of the forages were made by Messrs. Anselmo Campos, Ricardo Bressani and Ruben Oro of INCAP.

### REFERENCES

- Bessey, O. A., O. H. Lowry, M. J. Brock and J. A. Lopez, 1946. Determination of vitamin A and carotene in small quantities of blood serum. J. Biol. Chem. 166: 177–188.
- Burch, H. B., O. A. Bessey and O. H. Lowry, 1948. Fluorometric measurements of riboflavin and its natural derivatives in small quantities of blood serum and cells. J. Biol. Chem. 175: 457–470.
- Cooney, W. T., J. S. Butts and L. E. Bacon, 1948. Alfalfa meal in chick rations. Poultry Sci. 29: 828-830.
- Davies, G. K., N. R. Mehrhof, C. L. Comar, L. Singer and K. Boney, 1947. Ramie meal a new feed. Annual Rept., U. Florida Agric. Exp. Sta.
- Goodland, R. L., R. R. Sealock, N. S. Scrimshaw and and L. C. Clark, 1949. Interference with the ultramicro ascorbic acid method of Lowry, Lopez and Bessey. Science, 109: 494–495.
- Lewy van Severen, M., and R. Carbonell, 1949. Estudios sobre digestibilidad de la pulpa de café y de la hoja de banano. El café de El Salvador, 19: 1619.
- Lowry, O. H., and T. H. Hunter, 1945. The determination of serum protein concentrations with a gradient tube. J. Biol. Chem. 159: 465-474.
- Lowry, O. H., J. A. Lopez and O. A. Bessey, 1945. Determination of ascorbic acid in small amounts of blood serum. J. Biol. Chem. 160: 609-615.
- Squibb, R. L., A. Falla, J. A. Fuentes and H. T. Love, 1950. Value of desmodium, pigeonpea fodder, Guatemalan and United States alfalfa meals in rations for baby chicks. Poultry Sci. 29: 482-485.
- Squibb, R. L., and E. Salazar, 1951. Value of corozo palm nut and sesame oil meals, bananas, APF, and cow manure in rations for growing and fattening pigs. J. Animal Sci. 10: 545-550.
- Squibb, R. L., and M. K. Wyld, 1950. Further studies on the value of desmodium meal in the baby chick diet. Poultry Sci. 29: 586-589.
- Squibb, R. L., and M. K. Wyld, 1952. Effect of corozo palm nut oil meal in the baby chick diet. Poultry Sci. 31: 118-122.