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Utilization of the Carotenoids of Bamboo Leaves, Teosinte and Ixbut by New Hampshire Chicks

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AS A RESULT of the low vitamin A intake during extended dry seasons, vitamin A deficiency is a severe nutritional problem of animals in the American tropics. It is now apparent that not only inadequate dietary intakes but also high environmental temperatures (Squibb, Guzmán and Scrimshaw, 1954) and, in poultry at least, diseases such as coryza and cholera (Squibb *et al.*, 1955) are additional factors responsible for low serum vitamin A levels in animals. All of these are common stress factors in the tropics.

Squibb, Guzmán and Scrimshaw (1953, a, b, c) and Squibb, Méndez, Guzmán and Scrimshaw (1954) have demonstrated that vitamin A deficiencies may be overcome by feeding dehydrated forage meals prepared from kikuyu grass (*Pennisetum clandestinum*), ramie (*Boehmeria nivea*), *Desmodium sp.*, especially the species *D. intortum*, or banana leaves. In this report the utilization of the carotenoids of four additional forages was evaluated in New Hampshire chicks. Data on the chemical content of the forages are also presented.

MATERIALS AND METHODS

Six-inch tips were cut from mature ixbut plants (*Euphorbia lancifolia*). Whole plants of teosinte (*Euchlaena mexicana*) were harvested at 12 inches in height. The 2 bamboo forages consisted of leaves which were obtained from a yellow (*Bambusa vulgaris*) and a green species (*Bambusa ventricosa*).[3] All were dehydrated by warm moving air which did not exceed 55°C. and then ground finely in a Wiley mill. The carotene pigments of representative samples were extracted with petroleum ether and passed through a magnesium oxide-celite column according to the method of Guilbert (1934) as modified by Peterson *et al.* (1937). The beta-carotene-containing fraction was eluted with 5% acetone in petroleum ether and read in a colorimeter at 440 mmμ. The methods employed for the determination of the other nutrients have been cited previously (Bressani *et al.*, 1953).

The forage meals were fed to 3-day-old New Hampshire straight-run chicks at 0.0, 0.1, 0.4 and 0.8%, replacing a part of the ground sorghum and cottonseed oil meal of a low vitamin A, all-vegetable protein basal ration. In gm. per 100 gm. of diet this ration contained: 30, sesame oil meal; 15, corozo oil meal; 4, cottonseed oil meal; 47.3, ground sorghum; 3, minerals including the minor elements and 0.7 of an aureomycin-vitamin B_{12} feeding supplement.[4] To each 100 gm. of this basal diet were added: 0.02 gm. vitamin D supplement;[5] 0.20 mg. thiamine chloride; 0.35 mg. riboflavin; 1.20 mg. calcium pantothenate; 1.50 mg. niacin; 0.35 mg. pyridoxine and 125.00 mg. choline chloride.

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[3] The bamboo leaves were collected and identified by Dr. Floyd A. McClure of the Smithsonian Institute.

[4] Courtesy of Dr. T. H. Jukes and the Lederle Laboratories.

[5] Delsterol, DuPont Company.

Previous studies indicated the carotenoid content of the basal ration to be negligible and that 100% mortality of baby chicks from avitaminosis A occurred before 42 days of age (Squibb, Guzmán and Scrimshaw, 1953b).

Nine to 12 chicks were housed in each all-wire cage and a total of 18 to 24 chicks were allotted to each group on the basis of weight. An appropriate temperature for the age of the birds was maintained thermostatically. Feed and water were provided *ad libitum* and all chicks were weighed individually each week. At the end of a 5-week feeding period each chick was bled by heart puncture and the serum analyzed for total carotenoids and vitamin A by the micro-method of Bessey *et al.* (1946).

RESULTS

The results of the chemical analyses of the four forage meals are shown in Table 1. Of particular interest for this study are the carotene contents, which were 15.05 mg./100 gm. for the ixbut sample, 12.32 mg./100 gm. for the yellow bamboo leaves, 8.61 mg./100 gm. for the green bamboo leaves, and 8.28 mg./100 gm. for the teosinte plant.

The number and grouping of the baby chicks, the percentage of forage meals fed, the growth, mortality and efficiencies of feed utilization are presented in Table 2, together with the blood sera data. It will be noted that 0.4% of ixbut, teosinte, and green bamboo leaves and 0.1% of yellow bamboo leaves were sufficient to prevent mortality in chicks on a vitamin A deficient diet. The greatest weight gains were observed in the groups receiving 0.8% of the forage meals with the exception of ixbut where no increase in weight gains was observed over the 0.4% level. The linear components of these weight gains were highly significant. The efficiency of feed utilization was improved with the addition of 0.1% to 0.8% of the four forage meals.

TABLE 1.—*Chemical composition of dehydrated forage meals prepared from ixbut, yellow and green bamboo leaves and teosinte*

Ingredients	Ixbut	Yellow bamboo leaves	Green bamboo leaves	Teosinte
Ash, gm.%	9.8	21.3	19.7	12.5
Calcium, mg.%	1,519	—	—	1,163
Iron, mg.%	53.3	13.4	8.0	147.6
Phosphorus, mg.%	400	86	86	320
Moisture, gm. %	9.2	8.6	6.6	4.0
Ether extract, gm.%	5.9	2.5	2.3	3.1
Crude fiber, gm.%	16.2	21.7	24.4	21.6
Nitrogen, gm.%	1.90	1.61	1.55	1.20
Carotene, mg.%	15.05	12.32	8.61	8.28
Vitamin C, mg.%	73	6	8	38
Vitamin B_2, mg.%	0.66	0.90	0.65	0.57
Vitamin B_1, mg.%	0.04	0.10	0.16	0.10
Niacin, mg.%	4.22	2.54	3.10	3.32

Serum vitamin A and carotene levels showed a significant linear response to increasing levels of these forages in the diet. Ixbut, which contained the greatest amount of carotene, maintained the highest serum vitamin A and carotene levels. Teosinte forage, on the other hand, containing the lowest carotene content as determined by the chemical method, maintained the second highest serum vitamin A and carotene levels. Dehydrated forage meals made from yellow and green bamboo leaves were intermediate in their effect on serum levels of these blood constituents.

DISCUSSION

Although the chemical analyses of the four forage meals showed them to be relatively low in protein content, they are excellent sources of vitamin A activity when properly prepared. This is indicated by the fact that on the average as little as 0.8% of any of the meals maintained adequate serum levels of vitamin A and carotene and supported good growth for the type of basal ration employed.

Within each of the forage meals there was a linear relationship between the carotene content in the diet, as determined by the chemical method, and the correspond-

TABLE 2.—*Mortality, weight gains and serum vitamin A and carotene levels of New Hampshire chicks fed a vitamin A deficient ration supplemented with four forage meals*

	Forage meal %	No. of birds	Mortality %	Final average weight,[1] gm.	Feed efficiency, gm./feed/gm. growth	Gain/bird/day, gm.	Carotene/gm. gain/day, mcg.	Serum	
								Vitamin A[1], mcg.%	Carotene,[1] mcg.%
Ixbut Plants									
	0.0	22	23	174	2.77	3.44	0.00	1	25
	0.1	24	13	191	2.57	4.38	0.38	4	45
	0.4	24	0	244	2.14	5.67	1.30	19	143
	0.8	23	0	240	2.52	5.62	3.02	32	286
Yellow Bamboo Leaves									
	0.0	23	48	198	3.25	3.79	0.00	2	22
	0.1	24	0	212	2.82	4.92	0.03	4	27
	0.4	23	0	214	2.91	4.95	1.43	10	71
	0.8	24	0	251	2.83	6.02	2.79	18	121
Green Bamboo Leaves									
	0.0	22	55	163	—	3.29	0.00	—	15
	0.1	21	19	204	3.07	4.77	0.26	3	27
	0.4	19	0	234	2.82	5.37	0.97	9	63
	0.8	24	0	252	2.76	6.05	1.90	10	100
Teosinte Plants									
	0.0	23	30	167	3.67	3.50	0.00	—	28
	0.1	24	13	190	2.45	4.64	0.02	7	37
	0.4	23	0	230	2.55	5.33	0.85	12	86
	0.8	24	0	248	2.44	5.88	1.62	24	146

[1] Linear components highly significant.

ing serum vitamin A and carotene levels of the chicks. The absolute effect on serum levels of a given carotene content, however, varied with the particular forage meal. The relative efficiencies of carotene absorption and the subsequent conversion of carotene to vitamin A among the different forages studied could not be predicted from chemical analysis alone. For example, ixbut contained the highest carotene content of the four forages, and as would be expected, it maintained the highest serum levels of vitamin A and carotene. Teosinte, however, which contained the least carotene, maintained higher serum vitamin A and carotene levels than those observed in chicks fed either of the two bamboo forage meals which by analysis contained more carotene.

The observed discrepancy in vitamin A activity of the carotene among the four forage meals reported here could be due to a number of causes. The carotene fraction measured by the chemical method is principally beta carotene, but any alpha and gamma carotene present would also have contributed to the total found by analysis without adding proportionally to the biological activity. Moreover, the method would not have determined any hydroxy carotene which might have been present and which might have added to the vitamin A activity. We have already pointed out that accurate estimations of the vitamin A activity of corn require the analysis of all the active carotene components which may be present along with the use of the proper factor for calculating their relative activity (Squibb, Bressani and Scrimshaw, 1957). The data indicate that forage meals must be analyzed similarly to estimate their true vitamin A activity from chemical analysis alone.

SUMMARY

The carotene content and vitamin A activity of dehydrated forage meals prepared from ixbut (*Euphorbia lancifolia*),

teosinte (*Euchlaena mexicana*), yellow bamboo leaves (*Bambusa vulgaris*) and green bamboo leaves (*Bambusa ventricosa*) were studied. Biological assays with New Hampshire chicks showed that dehydrated meals in the following amounts: 0.4% ixbut containing 15.00 mg. carotene/100 gm., 0.8% teosinte containing 8.32 and yellow and green bamboo leaves containing 12.32 and 8.61 mg./100 gm. respectively, prevented mortality and promoted good weight gains and serum vitamin A and total carotenoid levels in 5-week-old New Hampshire chicks fed vitamin A deficient rations.

The data illustrate the necessity of determining the nature and activity of the carotene present if the vitamin A activity is to be estimated accurately from chemical analyses.

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