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Carotene and Riboflavin Retention and Serum Vitamin Levels in Vitamin A Depleted Rats Fed Four Forage Meals, Achiote Meal, and African Palm Oil *

COMPENDIO

Cuatro tortas forrajeras, torta de achiote, y aceite de palma africana fueron suministrados a ratas de linaje USDA a las cuales se les había agotado la vitamina A. El porcentaje de absorción de carotenoides de las tortas forrajeras deshidratadas fué el siguiente: desmodium (Desmodium intortum), *57; pasto kikuyo* (Pennisetum clandestinum), *77; ramio* (Boehmeria nivea), *41; y torta de hojas de banano, 66. El porcentaje de absorción de la semilla entera de achiote* (Bixa orellana) *fué 44 y de aceite de palma africana* (Elaesis quineensis), *65. El porcentaje de riboflavina retenido por las ratas de los diversos alimentos fue: de desmodium, 77; pasto kikuyo, 84; ramio, 80; hojas de banano, 40; achiote, 69; y aceite de palma africana, 68. A pesar de las diferencias en absorción de carotenoides y riboflavina de los diferentes alimentos, no se presentaron diferencias significativas en los valores de suero de riboflavina o de vitamina A. Aunque los carotenoides del achiote fueron bien absorbidos, el alto contenido de suero de carotina indica que no todos los carotenoides, medidos químicamente, tienen actividad de vitamina A.*

DEHYDRATED desmodium (*Desmodium intortum*), kikuyu grass (*Pennisetum clandestinum*), ramie (*Boehmeria nivea*), and banana leaf forage meals are commonly available in the American Tropics and have been shown to have value as a source of carotenoids and riboflavin in baby chick rations by Squibb *et al* (5). Two other tropical products have excellent potentialities as sources of carotenoids in both animal and human feeding. African palm oil (*Elaesis quineensis*) and achiote (*Bixa orellana*). The carotenoids in African palm oil have been observed to cause carotenemia in human beings in Costa Rica [1]/ and those of achiote have been found to possess vitamin A activity (1). Achiote is widely used as a vegetable coloring agent.

The high efficiency of the rat in converting carotenoids to vitamin A and the fact that the conversion takes place in the intestinal wall makes the rat useful for absorption studies of the vitamin A activity of feeds. Sexton *et al* (3) could demonstrate no carotene in the livers of rats fed diets containing 25 percent alfalfa after a 14-day feeding period. Patel *et al* (2) have demonstrated that cryptoxanthin likewise does not appear in the liver of the rat unless injected intraperitoneally. In tests on the effect of diet on several blood constituents of animals Squibb *et al* (6) found less than 2 micrograms percent carotenoids in the blood serum of rats.

The present studies were designed to measure rats' absorption of carotenoids from forage meals made of dehydrated desmodium, Kikuyu grass, ramie, and banana leaf, and from African palm oil and achiote meal. The influence of these foods on 5 constituents of the blood serum and on the intake and retention of riboflavin also was determined from routine blood analyses.

Methods

Eighty weanling white rats first were fed a ration low in vitamin A: Crude casein, 20; sucrose, 63,98; U. S. P. No. XII salt mixture, 4; Wesson oil, 5; brewers' yeast, 7; and delsterol, 0.02. Preliminary tests indicated that weanling rats would become depleted of vitamin A in approximately 50 to 65 days on this diet and would average more than 150 grams in weight at the end of the period. A large rat was needed at the end of the period so that 2 to 3 ml. blood samples could be obtained readily by heart puncture.

Individual weights were recorded twice weekly in the early part of the depleting period; thereafter they were recorded at more frequent intervals. The rats were considered depleted of vitamin A, when, in a 7-day period, they either failed to gain or started to lose weight. All the rats showed some clinical symptoms of avitaminosis A in the eyes.

When the rats were depleted, they were divided into 6 comparable groups of 5 females and 5 males and housed in individual wire cages with raised screen floors. They were then fed diets that included one of the carotenoid-rich foods (desmodium, Kikuyu grass, ramie, and banana leaf forage meals; African palm oil; or achiote meal). Both the experimental diets and water were provided ad libitum. Blood samples were obtained before the trial began, from a representative group of 6 male and 6 female depleted rats, in order to establish

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1/ Personal communication of Dr. Antonio Peña Chavarría, 1951.

the carotenoid and vitamin A serum levels of depleted animals. These rats were then discarded.

Freshly cut desmodium, Kikuyu grass, ramie, and banana leaf forages were dehydrated in an oven with moving air at 115°F. These dried forages were finely ground, as was the whole seed of the achiote. Because of the presence of certain chick-growth-depressing factor or factors in desmodium meal (4), all the forages were fed to replace 5 percent of the sucrose of the basal diet, regardless of their carotenoid content.

Each group of rats was maintained on the test diet for 12 days. After the 5th day, the food intakes were carefully measured and the total feces collected and pooled for each group. Total carotenoids and riboflavin were determined on both the feeds and feces. These data then were used to calculate the rate of absorption of the carotenoids and retention of the riboflavin. The combined absorption and retention of these nutrients was calculated by subtracting the amount excreted in the feces from the amount consumed in the food. No corrections were made for possible intestinal synthesis of riboflavin.

At the end of the 12-day experimental period, 2 to 3 ml. of blood were obtained by heart puncture from each of the rats and the serum was analyzed for total proteins, riboflavin, ascorbic acid, alkaline phosphatase, carotenoids, vitamin A, and total tocopherols by chemical methods previously cited (6).

Results

The weight gains, efficiencies of feed utilization, and the percent carotenoids absorbed are presented in table 1. There were no differences in the rate of gain and efficiency of feed utilization among the rats fed one of the 4 dehydrated forage meals or the achiote. The group fed the palm oil, however, showed a depression of growth and feed utilization.

Blood analyses of the 12 rats used to determine the residual carotenoids and vitamin A following the depletion period showed their serums to contain only 1.0 microgram percent carotenoids and 1.1 micrograms percent vitamin A. The absorption data at the end of the subsequent 12-day period (table 1) showed that 59 percent of the carotenoids consumed were absorbed if desmodium was fed, 77 percent if Kikuyu grass, 41 percent if ramie, and 66 percent if banana leaves. If palm oil was fed, 65 percent was absorbed; if achiote, 44 percent.

The levels of serum vitamin A were similar in the groups fed the forage meals and the palm oil. The group fed achiote had a significantly lower level of this nutrient. Serum carotenoids were greatly elevated in the group fed achiote but low in the groups fed the kikuyo grass, ramie, and desmodium forage meals. The serum carotenoid levels of the groups fed the banana leaf meal and palm oil were significantly higher than those of the rats fed the other forage meals.

The percent retention of riboflavin was lowest in the group fed banana leaves (table 2), which received the highest amount of riboflavin in the diet. The group fed the palm oil had the lowest riboflavin intake and retained the least amount although its percent retention was the same as that of the group fed achiote and desmodium. The groups fed ramie and kikuyu grass had an intermediate riboflavin intake and a relatively high absorption. The resulting riboflavin serum levels were not significantly different among the six groups, but the highest riboflavin levels were found in the groups fed desmodium and banana leaves.

The serum levels for the other nutrients are given in table 3. Total tocopherols were significantly higher in the serum of the rats fed ramie and banana leaf forages and achiote meal. Of these, the ones fed banana leaves maintained the highest level of tocopherols. The al-

Table 1. Absorption of carotenoids and the resulting levels of carotenoid and vitamin A in blood serum, for rats fed four forage meals, achiote, and African palm oil.

INGREDIENTS TESTED	Number of rats	Weight gained	Feed per gram gain	TOTAL CAROTENOIDS			BLOOD SERUM	
				Intake	Excretion	Absortion	Carotenoids	Vitamin A
		gms.	gms.	mg.	mg.	%	γ/%	γ/%
Check group	12	—	—	—	—	—	1	1.1
Forage meals:								
Desmodium	10	36	4.28	13.68	5.56	59	3	23.1
Kikuyu grass	10	36	4.10	17.41	3.94	77	4	23.6
Ramie	10	33	4.60	9.77	5.76	41	4	25.5
Banana leaves	10	38	4.65	13.60	4.65	66	8	19.2
Other feeds:								
Palm oil	10	26	5.41	17.95	6.24	65	6	20.5
Achiote meal	10	33	4.56	1.42	0.79	44	35	11.9

Least significant difference at 5 percent level: for serum carotenoids, 3.0; for vitamin A, 7.6.

kaline phosphatase was significantly lower in the group fed the achiote meal. Other variations in alkaline phosphatase were not significant. Total protein and ascorbic acid serum levels did not differ significantly among the groups.

Discussion

Of the 6 feedstuffs studied, the ones that resulted in highest absorption of carotenoids were the ones that furnished highest total intake. For all groups of rats except the one receiving achiote, the serum carotenoids remained low, indicating good conversion to vitamin A. Apparently, either the rat cannot convert some of the carotenoids of achiote, as determined chemically, into vitamin A, or some factor in achiote itself interferes with this conversion. These high serum carotene values in rats fed achiote appear all the more extraordinary in view of the fact that the total intake of carotenoids by the rats fed achiote was only 8 to 14 percent that of the other groups. Nevertheless, the vitamin A level of this group was 10 times that of the control group and approximately half that of the groups receiving one of the forage meals or the palm oil. These data indicate that some of the carotenoids of achiote must possess good vitamin A activity. These data are in agreement with the findings of Cook and Axtmayer (1).

Table 2. Intake and retention of riboflavin in rats fed four forage meals, achiote, and african palm oil.

INGREDIENTS TESTED	RIBOFLAVIN			
	Total intake in feed	Total retained in body 1/		Serum 2/
	mg.	mg.	%	γ/%
Forage meals:				
Banana leaves	8.15	3.29	40	4.22
Kikuyu grass	4.87	4.07	84	3.69
Desmodium	4.66	3.60	77	4.35
Ramie	4.19	3.36	80	3.53
Other feeds:				
Achiote meal	7.11	4.92	69	3.20
Palm oil	3.34	2.08	68	3.05

1/ Calculated by subtracting total excreted in feces from total intake in feed. No correction was made for possible intestinal synthesis.
2/ Least significant difference at 5 percent level: serum riboflavin, 1.04.

Table 3. Effect of four dehydrated forage meals, achiote, and African palm oil on four blood serum constituents of rats.

INGREDIENTS	Total proteins	Ascorbic acid	Alkaline phosphatase	Total tocopherols
	mg/%	mg/%	Units 1/	mg/%
Forage meals:				
Kikuyu	6.40	0.73	4.08	0.04
Desmodium	6.30	0.92	5.28	0.03
Ramie	6.35	0.93	6.06	0.16
Banana leaf	6.20	0.85	4.74	0.31
Other feeds:				
Achiote	6.81	0.75	2.92	0.16
Palm oil	6.29	0.79	5.24	0.09
Least significant difference at 5-percent level	0.46	0.17	2.19	0.12

1/ Millimoles per liter per hour.

The fact that the other differences in carotene intake failed to be reflected in divergent vitamin A values may have been due partly to the relatively short duration of the experiment. However, the rate of storage of this vitamin and the rate of conversion of the carotenoids to vitamin A in the intestinal wall may have tended to compensate for the differences in intake as well as to account for some of the differences in absorption.

Since the rats were not depleted of riboflavin, the retention of riboflavin might be expected to be influenced by differences in total intake. This correlation between retention and intake appeared to apply to all groups of rats except the one fed banana leaves, which had the highest intake but showed the lowest percent retained. Although the group fed palm oil had the lowest intake of riboflavin, the percent retained was similar to that of the group fed achiote, which had nearly twice the riboflavin intake.

No explanation can be given for the relatively similar serum riboflavin levels in the various groups of rats. Intestinal synthesis, interferring dietary factors, or possible differences in the rate of utilization and storage could have been influencing factors.

The significant differences among the serum alkaline phosphatase values of the different groups likewise cannot be explained by the data of this experiment. The high serum tocopherol levels of the rats fed banana leaf and ramie forage meals and achiote appear to be due to the high levels of tocopherols in these feeds.

Summary

Four forage meals, achiote meal, and African palm oil were fed to vitamin-A-depleted rats of the USDA strain. The percent absorption of carotenoids from the dehydrated forage meals was as follows: Desmodium (*Desmodium intortum*), 57; kikuyu grass (*Pennisetum clandestinum*), 77; ramie (*Boehmeria nivea*), 41; and banana leaves, 66. Percent absorption from the whole seed of achiote (*Bixa orellana*) was 44 percent; from oil from the African palm (*Elaesis quineensis*), 65 percent.

The percent riboflavin retained by the rats from the different feedstuffs was as follows: Desmodium, 77; kikuyo grass, 84; ramie, 80; banana leaves, 40; achiote, 69; and African palm oil, 68.

Despite differences in the total intake of carotenoids and riboflavin from the forage meals and from the African palm oil, there were no significant differences in serum riboflavin or vitamin A values. Possible explanations for these phenomena are suggested.

Although the carotenoids of achiote were well-absorbed, the high serum carotene value shows that not all the carotenoids, as measured chemically, possessed vitamin A activity.

It is concluded that the dehydrated forages, achiote, and African palm oil are all good sources of vitamin A activity for rats.

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ROBERT L. SQUIBB
INSTITUTO AGROPECUARIO NACIONAL
GUATEMALA CITY, GUATEMALA.

MIGUEL GUZMAN, CHIEF, MICRO-ANALYSIS SECTION
INSTITUTO DE NUTRICION DE CENTRO AMERICA Y PANAMA. (INCAP)
GUATEMALA CITY, GUATEMALA.

NEVIN S. SCRIMSHAW, DIRECTOR
INSTITUTO DE NUTRICION DE CENTRO AMERICA Y PANAMA
GUATEMALA CITY, GUATEMALA AND REGIONAL ADVISER IN NUTRITION, PAN AMERICAN SANITARY BUREAU, REGIONAL OFFICE FOR THE AMERICAS OF THE WORLD HEALTH ORGANITATION.