

## THE VITAMIN A CONTENT OF AVOCADOS\*

**Leroy S. Weatherby, J. Ellsworth Youtz, and Ruth V. Watson**

*Chemical Laboratory, University of Southern California*

The avocado is distinctive among fruits in that it is practically the only fruit consumed fresh in which the food supply is stored largely as an oil. This makes possible the storage of fat-soluble vitamins. The conditions under which the avocado grows, with heavy foliage under a semitropical sun, create favorable conditions for vitamin synthesis. This report covers the determination of vitamin A (1).

Since the quantity of oil present presumably depends on the oil content of the fruit, determinations were made on the Calavo, the highest grade of avocado in which the fruit must meet certain high standards of maturity and of oil content. Since vitamin A is susceptible to oxidation, all determinations were made on fresh fruit which was furnished through the courtesy of the Calavo Growers of California. The Fuerte variety was, with few exceptions, used throughout the tests.

The determinations were made through feeding experiments by the curative methods with albino rats. The cages used had raised screen bottoms to prevent access to excreta.

In the preliminary experiment comparison was made of rats on normal diet, vitamin-A-free diet, and avocado diet using 5 gms. of avocado per day. The normal diet used was as follows:

Meat residue, 20 per cent	Butter, 5 per cent
Starch, 52 per cent	Salts (McCullum's No. 185) (2), 4 per cent
Lard, 19 per cent	Yeast, 1 gm. per rat per day

The meat residue was prepared by extracting freshly ground round steak three times with hot water and three times with hot alcohol, and drying at a moderate temperature. Kingford's corn starch was used untreated in this first series. The butter was unsalted sweet cream butter. The lard was later replaced by Crisco. The yeast was Fleischmann's dry yeast.

In the vitamin-A-free diet, the butter was replaced by an equivalent amount of lard. In the avocado diet the butter was omitted and fresh avocado pulp was added to the vitamin-A-free diet daily.

In this series, on the diet used it required about 55 days for depletion of vitamin A. Graph 1 gives characteristic growth curves showing the effect of 5 gms. of avocado under these conditions. The negative control animals showed all symptoms of A-avitaminosis, with severe ophthalmia, and showed on autopsy the characteristic pus sacks at base of tongue and under cheeks and also frequently in the lungs and other

vital organs. No animal on avocado diet showed these symptoms. The rat shown in the graph on the deficiency diet had reached the state of ophthalmia in which its eyes were swollen practically shut, but these symptoms disappeared in five days on the addition to its diet of 5 gms. per day of avocado.

These preliminary experiments indicated definitely the presence of vitamin A in avocados in considerable quantity. It was followed, therefore, by an exact quantitative determination.

In the second series of tests, a quantitative determination was made using younger animals with less opportunity for vitamin storage and with a more rigorous vitamin A free basal diet.

Rats from two litters weaned at 30 days, weighing about 50 gms. each were placed directly on the vitamin-A-free diet which was a modification of the Osborne and Mendel control diet, and which was made up as follows:

Casein (alcohol extracted), 18 per cent	Salt mixture (McCollum's No. 185), 4 per cent
Starch (alcohol extracted), 54 per cent	Sodium iodide, 0.001 of the salt mixture
Lard (aerated), 24 per cent	Yeast (Fleischmann's dry), 1 gm. per rat per day

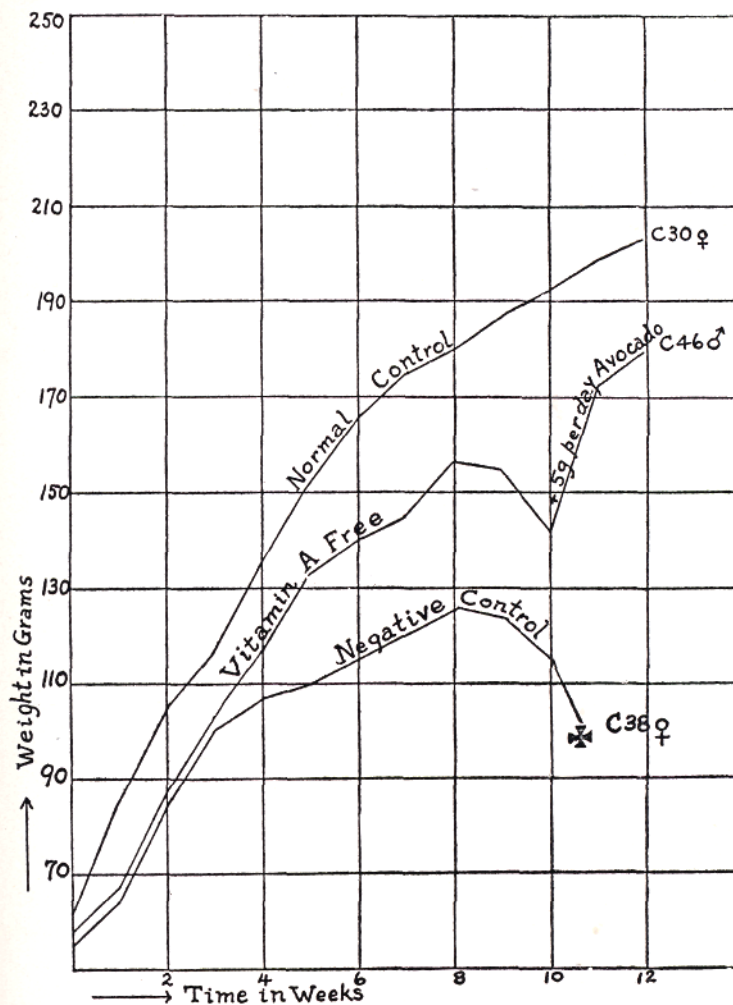
The casein used was edible casein (muriatic) no. 453 obtained from the Casein Manufacturing Company, 15 Clark Row, New York. In order to remove all traces of vitamin A, this was extracted three times by refluxing 3 hours each time with 95 per cent alcohol. It was filtered by suction each time and was finally dried at a low heat. The starch was Kingsford's corn starch which was in like manner extracted for 1 hour with 95 per cent alcohol. The lard was melted and held at 100 C. while a current of air was passed through for 3 hours. The pulp of the ripe avocado was prepared daily and put through a sieve. This with the yeast was placed on top of the food in the food cups and was eaten greedily without waste.

In order to compensate for vitamin D deficiency and protect against rickets, all the animals were exposed to the direct rays of the sun two days per week between the hours of 12 and 3 o'clock.

The food test groups were arranged with three animals comprising each group with due regard to distribution of size and sex as follows:

	Avocado per day grams		Avocado per day grams
Group 1, Negative control	0	Group 3- Medium avocado—	5.0
Group 2. Low avocado	2.5	Group 4. High avocado	7.5

On the assumption of 20 per cent oil content, this would be equivalent to 0.5 gm., 1 gm., and 1.5 gms. oil respectively.



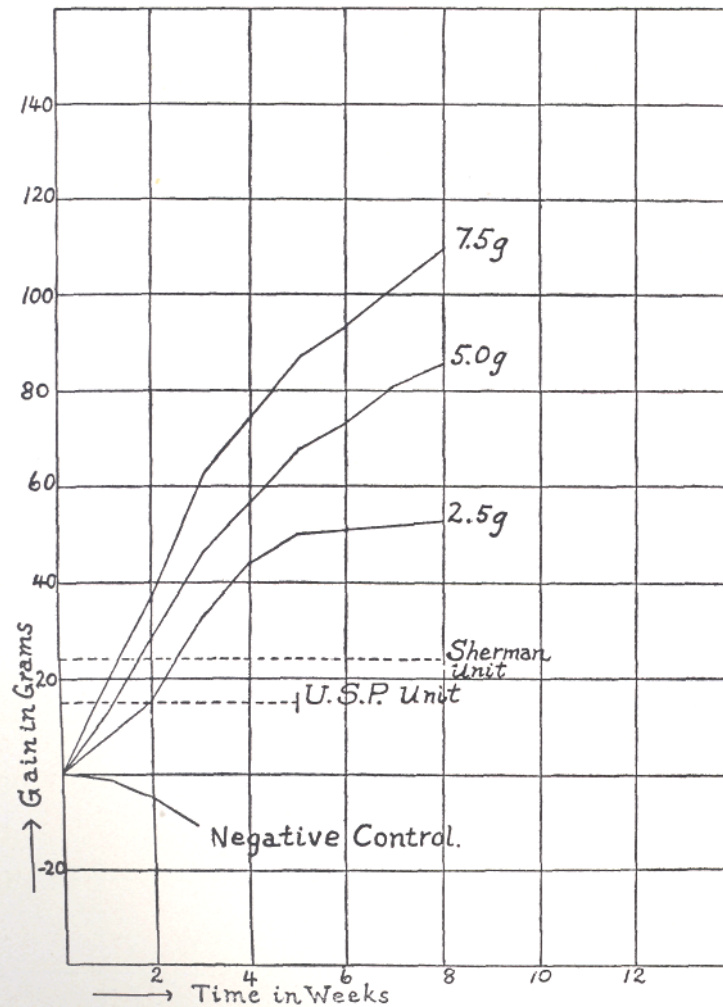
GRAPH 1. GROWTH CURVES OF RATS ON DIETS PLANNED TO DETERMINE THE PRESENCE OF VITAMIN A IN AVOCADOS

On the assumption of 20 per cent oil content, this would be equivalent to 0.5 gm., 1 gm., and 1.5 gms. oil respectively.

In order to keep the fat content of the diet constant, an equivalent amount of lard was omitted when avocado pulp was added, calculating the avocado as 20 per cent oil. In the high avocado diet, the greater part of the fat was that of the avocado.

Under the conditions of this series, depletion occurred in about five weeks as indicated both by the presence of ophthalmia and by loss in weight. After the sixth week, the animals were placed on the test diets. One of the negative control animals died during the second week of the test period and the remaining two during the third week. The animals placed on avocado diet soon lost all symptoms of ophthalmia and had all appearance of normal animals. Graph II gives the average growth curve for the group during the food test period.

The growth curves show that the vitamin A content is even higher than had been anticipated from the preliminary work. Inspection of the curves shows that the quantity of avocado necessary to maintain an average growth of 3 gms. per week during an eight week test period, the standard suggested by Sherman (3) for the unit of vitamin A would be considerably less than the smallest quantity used, 2.5 gms. Based on the United States Pharmacopoeia unit (4) for vitamin A which calls for a gain in weight of from 10 to 20 gms. within a period of thirty-five days only, the quantity of avocado required would be less than that required for the Sherman unit.



GRAPH 2. GROWTH CURVES OF RATS ON CONTROL DIET AND ON DIETS CONTAINING DIFFERENT QUANTITIES OF AVOCADOS

During the depletion period, four animals of like sex were kept in the same cage. During the food-test period they were kept in individual cages.

The results of this investigation show the avocado, as predicted, to be high in vitamin A content. It should be remembered, however, that these investigations were carried on with fruit of Calavo grade, meeting the highest standards of maturity and of oil content, which are probably important factors in governing the vitamin content.

## References

1. Weatherby, L. S. and Waterman, E. W. Vitamin B. Content of Avocados. *Ind. and Eng. Chem.* 20, 968-970 (1928).
2. McCollum E. V. Study of Dietary Essential Water Soluble B in Relation to its Solubility and Stability toward Reagents. *J. Biol. Chem.* 33, 55-89 (1918) table p. 63.
3. Sherman, H. C. *Chemistry of Food and Nutrition*. Third edition. New York: The Macmillan Company, 1926, p. 456.
4. U. S. Pharmacopoeia X, p. 469.