8-1936

The Effects of Vitamins "A" & "D" Deficient Diets on Crayfish

Joseph Hale
Western Kentucky University

Hale,

Joseph Flexner

1936

THE EFFECTS OF VITAMINS "A" AND "D" DEFICIENT DIETS ON CRAYFISH

BY

JOSEPH FLEXNER HALE

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ARTS

WESTERN KENTUCKY STATE TEACHERS COLLEGE

August, 1936

Approved:-

Major Professor L. W. Sonneborn

Department of Biology M. C. Ford

Minor Professor J. T. Stinson

Graduate Committee

CONTENTS

TABLES

PURPOSE OF THE STUDY

The literature on vitamins shows that the greater number of the studies made of vitamin "A" and "D" deficient diets have been confined to their efforts on the vertebrates. Only a few controlled experiments have been conducted to determine the effects of vitamin "A" and "D" deficient diets on the invertebrates.

The purpose of this investigation was to determine the effects of vitamin "A" and "D" deficient diets on crayfish.

REVIEW OF THE LITERATURE

The beneficial effect of fish-liver oils in the treatment of rickets, generalized malnutrition, and certain eye conditions were widely recognized by the middle of the nineteenth century. However, the superiority of these oils over other fats for dietary purposes was not definitely shown before the middle of the twentieth century when fish oils were demonstrated to contain a factor essential for nutrition. This constituent, which resisted saponification, could not be transferred to oils which formerly did not contain it.

McCollum[1] showed that cod-liver oil aerated at 100 degrees C. for several hours retained much of its anti-rachitic properties, but lost its growth-promoting powers entirely.

The presence of certain vitamins in the diets of animals becomes increasingly important in the ascending scale of animal life. However, information concerning the necessity of vitamins for the lower animals is not well developed.

---

1 Harrow and Sherwin, A Text-book of Biochemistry (Philadelphia, W. B. Saunders Co., 1935), p. 267.

An examination of the literature on vitamin "A" and "D" deficiencies in invertebrates showed that some work had been done with class Insecta of the phylum Arthropoda. The following table shows the known vitamin requirements for various animal groups in which vitamin experiments have been conducted.

TABLE I
Vitamin Requirements for Animal Groups

Animal	Vitamin
Fly (Drosophila)[A]	B
Flour beetle[B]	A, B
Cockroach[C]	A, B
Tadpole and frog[D]	A, B
Fish[E]	A, B
Bird[F]	A, B, C
Mammal[G]	A, B^1, B^2, C, D, E

Flour beetles have been shown to thrive on flour only when a little of the bran or germ is left in it. Flour moth larvae are unable to grow in the absence of a fat-soluble substance found in the wheat germ, egg yolk, and butter fat. The deficiencies are assumed to be "A" and "B".[1]

A
Pryde, John, Recent Advances in Biochemistry (Philadelphia, P. Blakinstons Sons and Co., Inc., 1931), pp. 261-262.

B
Williams, R. J., An Introduction to Biochemistry, (New York, D. Von Nostrand Co., Inc., 1931), p. 181.

C
Moskalenko, V., "Cockroaches a Test Animals in the Quantative Analysis of Vitamins in Food Stuffs," Chemical Abstracts, Vol. 28, No. 16, October, 1934), pp. 5101.

D
Pryde, John, op. cit., p. 262.

E
Ibid.

F
Ibid.

G
Ibid.

1
Williams, R. J., op. cit., p. 181.

2
Moskalenko, V., op. cit., pp. 5101.

3
Williams, R. J., op. cit., p. 186.

Maskalenko reports vitamin "C" is not essential for development of cockroach nymphs which require the vitamin "A" and "B" complex, the necessary part of the latter being probably vitamin "B". As a source of fat-soluble factors an extraction of wheat germ is most important.[1]

CHOICE AND GROUPING OF EXPERIMENTAL ANIMALS

Williams[2] states that the animal which is to be used for nutritional studies must be readily kept in captivity, hardy, and must be fed on a variety of diets. Extensive studies of the experimental animals should also yield a number of facts with reference to the nutrition of the large group of animals of which it is a representative.

Crayfish of the genus Cambarus were selected for this study on invertebrate nutrition because they were believed to meet most of the requirements for a good experimental animal. They are readily available, fairly hardy, and are omnivorous feeders.

After specimens were collected it was found that they were not uniform in size. Successful experimentation demands that the animals be of uniform size. The animals were therefore divided according to size. The smaller ones were thought to be best for testing growth with the vitamin "A" diet. The larger ones were used on a vitamin "D" deficient diet to test for calcium deficiency. Each group was subdivided. From each group fourteen specimens were put on a deficient diet, and fourteen on a control diet.

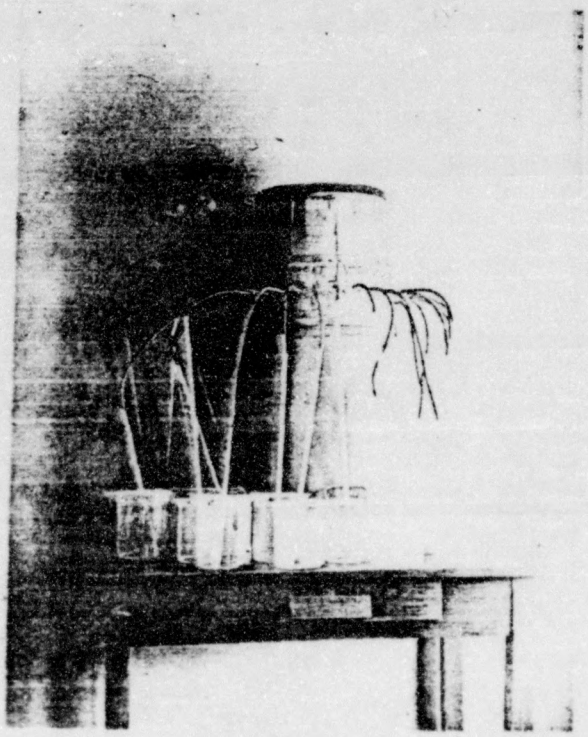
The group of young crayfish averaging 3.2 grams each were put on the vitamin "A" diets. The older ones averaging 11.1 grams were put on the vitamin "D" diets.

---

1 Moskalenko, V., op. cit., pp. 5101.

2 Williams, op. cit., p. 186.

FIGURES



DESCRIPTION OF APPARATUS

Animals fed on each diet required separate containers to prevent the mixing of foods and to avoid the effects due to loss of individuals. The specimens being of variable size necessitated keeping them separate, so replacements could be made if deaths occured during the feeding period. These conditions were provided for by using one liter beakers divided into two separate compartments by a partition made of hardware cloth. Seven beakers in this way served as fourteen individual aquaria which constituted a feeding unit. Four groups of seven beakers provided individual containers for fifty-six specimens. A set of fourteen specimens were fed on vitamin "A" control diet and another set of fourteen were fed "A" deficient diet. In like manner groups fourteen specimens each were fed on vitamin "D" control and "D" deficient diet.

Crayfish are usually found living in running water, so provisions were made to duplicate this condition in the laboratory by having flowing water in the aquaria supplied from a central reservoir. The central reservoir was a wooden bucket which held a reserve water supply. Twenty-eight holes spaced an even distance from the bottom of the reservoir, were fitted with short glass tubes. Pieces of rubber tubing were fitted on the short glass tubes at the reservoir and each was provided with an adjustable clamp to regulate the flow of water. Longer pieces of glass tubing were passed through the gauze covers on the beakers to conduct the fresh supply of water to the bottom of the aquaria. The bottom of the reservoir was twenty-one inches from the top of the aquaria affording a sufficient pressure to keep water steadily flowing through the conducting tubes.

The temperature of the water in the central reservoir varied from 12 to 14 degrees C. which was sufficiently high for the crayfish to be active.

DIETS USED

The feeding of aquatic animals present problems more complex than are encountered in case of non-aquatic forms, the major ones being the prevention of the dissolving out of nutrient materials in the food and a disintegration into particles too small for the crayfish to use. This was prevented by making a starch matrix for the food after it had been well ground in the mortar and thoroughly mixed with the hands.

Quantities sufficient for two feedings were prepared and spread in thin layers over large watch glasses. The food, after drying in an electric oven at 37 degrees C. was in a tough, compact form that resisted dispersion in the aquaria. These layers were broken up into small pellets which were found to be palatable to the crayfish.

The experimental animals were fed regularly twice a week over a period of eight weeks on the diets shown in TABLES II to V inclusive.

TABLE II
Vitamin "D" Deficient Diet---Steenbock 2965

Weight based on 100 grams.

Whole yellow corn meal (Vitamin A).....	76.0 grams
Wheat gluten.[A]	20.0
Calcium Carbonate.....	3.0
Sodium Chloride.....	1.0

TABLE III
Vitamin "D" Complete Diet---Steenbock 2968

Ninety-nine grams of the vitamin "D" deficient diet and one gram cod-liver oil.[A]

[A] Pure Gluten Food Co., High Bridge, N. J.

[A] United Drug Co., Boston, Mass. (Each gram of cod-liver oil contains more than 2100 units of vitamin "A" and more than 250 units of vitamin "D").

TABLE IV
Vitamin "A" Deficient Diet---College Heights

Weight based on 100 grams	
Casein II [A] (Vitamin Free)	18.0 grams
Brewer's Yeast Flakes	8.0
Salt 40	4.0
Composition of Salt 40	
NaCl	2.33 parts
$MgSo^4 \cdot 7\ H^2O$	2.46
$Na^2HPO^4 \cdot 12H^2O$	3.58
K^2HPO^4	6.96
$Ca^2H^2\ (PO^4)^2 \cdot 4\ H^2O$	6.88
Calcium Lactate	1.54
Iron Citrate	.594
KI	.016
Starch dextrinized	67.5
Cod-liver oil aerated at 100 degrees for 12 hours	1.0

TABLE V
Vitamin "A" Complete Diet

Ninety-nine grams of vitamin A deficient Diet and one gram non-aerated cod-liver oil

METHOD OF STUDY

Mammalian mal-nutrition in vitamin "A" is evidenced by a lack of growth, and eye afflictions. Crayfish were fed on vitamin "A" deficient diet to determine the effects of this growth promoting factor.

Young crayfish were weighed accurately to one-tenth gram when placed on their respective diets. At the end of the experimental period of eight weeks, weights were taken in order to determine the effect of vitamin "A" as a growth-promoting factor.

Representative specimens were prepared for a calcium determination at the same time that others were put on the vitamin "D" diets. At the end of

---

[A] Casein Mfg. Co., of America, Inc., 350 Madison Ave., N. Y., N. Y.

the feeding period, representative specimens were chosen from the vitamin "D" deficient and the vitamin "D" complete diets for calcium determinations. Calcium was extracted from the crayfish in HCl solution. The per cent of calcium was determined volumetrically by the calcium oxalate method.

RESULTS

The experimental results on the vitamin "A" complete and deficient diets, and the vitamin "D" complete and deficient diets are represented in TABLES VI to X inclusive.

TABLE VI
Results of Vitamin "A" Complete Diet

Specimen	Weight March 28	Weight May 15
1	5.1	5.4
2	4.0	4.3
3	2.2	2.3#
4	4.3	4.6
5	2.2	2.3
6	2.0	2.0#
7	3.0	3.1
8	2.2	2.3
9	4.2	4.2
10	3.8	3.9
11	1.6	1.4#
12	2.6	2.8
13	3.8	4.1
14	4.0	4.2

Replacements May 3.

TABLE VII
Results of Vitamin "A" Deficient Diet

Specimen	Weight March 28	Weight May 15
1	3.9	3.9
2	2.3	2.2#
3	3.7	3.7
4	3.7	3.8
5	4.8	4.8
6	4.8	4.7
7	5.3	5.3
8	3.5	3.4
9	3.3	Deaths no replacement
10	4.1	" " "

TABLE VII (Continued)
Results of Vitamin "A" Deficient Diet

Specimen	Weight March 28	Weight May 15
11	6.2	6.1
12	3.6	3.6
13	2.6	2.5
14	3.2	3.3

Replacement May 3

TABLE VIII
Determination of Calcium in Vitamin "D"
Control Specimens at the Beginning of
the Experiment

Amount of Ash in a representative size crayfish..... .931 grams

Dry weight of five representative specimens.....10.610

Per cent of calcium based on dry weight..... 5.780

Per cent of calcium in the mineral content.....13.770

TABLE IX
Determination of Calcium in Vitamin "D"
Deficient Specimens after Eight Weeks.

Amount of Ash in a representative size crayfish.... .896 grams

Dry weight of five representative specimens..... 9.820

Per cent of calcium based on dry weight..... 5.320

Per cent of calcium in the mineral content..... 13.100

TABLE X
Determination of Calcium in Vitamin "D"
Control Specimens after Eight Weeks.

Amount of Ash in a representative size crayfish.... .913 grams

Dry weight of five representative specimens.....9.740

Per cent of calcium based on dry weight.....5.270

Per cent of calcium in the mineral content.....13.360

SUMMARY

This investigation was undertaken for the purpose of determining the effects of vitamin "A" and "D" deficient diets on crayfish.

It was found in the specimens fed on a vitamin "A" deficient diet that a pronounced lack of growth resulted and in some cases loss of weight was recorded. Five of the fourteen specimens lost weight. Only two crayfish showed indications of slight gain, whereas four showed no change in weight. Results were not recorded on the two specimens which died. The total of the weight changes resulted in an actual loss of weight when crayfish were fed on a diet deficient in vitamin "A". The specimens on the vitamin "A" complete diet had an aggregate increase in growth of 2.1 grams in contrast to an aggregate loss of .6 grams in specimens on a vitamin "A" deficient diet.

The results from the experiments of the effect of vitamin "D" on the metabolism of crayfish, were not sufficiently pronounced to warrant definite conclusions. It is a well known fact that vitamin "D" is associated with calcium metabolism in the vertebrates and tests were conducted to determine if this is true for the crustacea. Calcium determinations were run on control specimens and on those fed a diet containing vitamin "D". The differences in amounts of calcium vary but little on control and vitamin "D" deficient diets.

The results would probably have been different had it been possible to keep the animals under the experimental conditions through several molts, because it is known that a large part of the calcium content of their bodies is in the exoskeleton. Further tests will be necessary before final decisions can be made.

BIBLIOGRAPHY

Harrow and Sherwin, A Text-book of Biochemistry (Philadelphia, W. B. Saunders Co., 1935).

Moskalenko, V., "Cockroaches a Test Animals in the Quantative Analysis of Vitamins in Food Stuffs," Chemical Abstracts, Vol. 28, No. 16 (October, 1934), 5101.

Pryde, John, Recent Advances in Biochemistry (Philadelphia, P. Blakinstons Sons and Co., Inc., 1931).

Williams, R. J., An Introduction to Biochemistry, (New York, D. Von Nostrand Co., Inc., 1931).