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7. Vitamins Essential for Growth of Channel Catfish

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Research was conducted at Auburn University, Auburn, Alabama

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VITAMINS ESSENTIAL FOR THE GROWTH OF CHANNEL CATFISH, *Ictalurus punctatus*

By Harry K. Dupree, Fishery Biologist
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Abstract.--Symptoms were identified in channel catfish fed diets deficient in the water-soluble vitamins pyridoxine, pantothenic acid, riboflavin, thiamine, folic acid, nicotinic acid, B-12, or choline. Fat-soluble vitamin A and vitamin K deficiency symptoms were observed after feeding diets which contained beta-carotene and 4.0 milligrams of menadione (synthetic vitamin K) per 100 grams of food (dry weight). These deficiencies were eliminated by substituting vitamin A palmitate for beta-carotene and doubling the content of menadione. No dietary need was observed for the water-soluble vitamins biotin, inositol, ascorbic acid, and para-aminobenzoic acid.

Soon after the recognition of the importance of vitamins in human nutrition, fishery workers became interested in the importance of these organic substances in the diets of fish. Jewell, Schneberger, and Ross (1933) conducted the only known research on the vitamin requirement of channel catfish. This early research was hampered by lack of information about the total number and identity of the vitamins and lack of a satisfactory vitamin test diet.

The increasing importance of this species in our commercial and sport fisheries demands considerable research on the nutritional requirements of channel catfish. Consequently, studies were designed to ascertain the specific water-soluble vitamin requirements for these catfish.

METHODS

The experiment was conducted, in three phases, at the Farm Ponds Laboratory of Auburn University, Auburn, Ala. The first phase was conducted during a 24-week period starting in May 1957, the second during a 30-week period starting in April 1958, and the last during a 36-week period starting in January 1959.

Twenty stainless steel troughs, each 7 feet by 1 foot by 0.8 foot deep, were available for the research. Each trough was equipped with water supply, air supply, and a standpipe drain. The residual chlorine in the water, from the city of Auburn's filtered and chlorinated domestic water supply, was removed with an activated-carbon filter. The rate of water flow through the troughs was approximately 0.2 gallon per minute.

The channel catfish fingerlings were obtained from rearing ponds of the Farm Ponds Project, Auburn University, Auburn, Ala. The fish were carefully examined for parasites and disease; if either was found, an appropriate treatment (Allison, 1957, and Van Duijn, 1956) was applied.

After the fish were stocked into the laboratory troughs and trained to eat a canned diet (3 to 10 days), the vitamin-complete diet was

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This research was conducted at Auburn University, Auburn, Ala., as a partial requirement for the degree of Doctor of Philosophy.

substituted and fed to the fish for approximately 2 weeks before the start of each phase.

The vitamin-complete and the vitamin-deficient diets were gelatin-bound, high-protein diets that contained 25 percent solid matter (Halver, 1957). These diets were prepared by mixing 99 grams of the purified basic nutrients (vitamin-free casein, gelatin, white dextrin, corn oil, salt mixture, and cellulose flour) with 1 gram of the appropriate vitamin supplement (in dextrose) in 300 milliliters of warm water (100° - 110° F.). The "complete" vitamin supplement contained the 12 water-soluble and the 4 fat-soluble vitamins in purified form. A vitamin-deficient supplement had 1 vitamin deleted. After blending for 5 minutes with an electric mixer, the thick, homogeneous mixture was chilled for 12 to 24 hours at temperatures near 45° F. The gelatinous mass was passed through a food grinder with 3/16-inch holes, and the extrusions were cut into 1/2- to 2-inch lengths.

In phase I of the experiment, the vitamin-complete test diet and the pyridoxine-, pantothenic acid-, thiamine- and riboflavin-deficient diets were similar to those described by Halver (1957) except that 4.0 parts of mineral mixture USP XIV (1950) were substituted for his mineral mixture. In phase II of the experiment the vitamin-complete test diet and the folic acid-, nicotinic acid-, biotin-, and inositol-deficient diets and in phase III the vitamin-complete test diet and the B-12-, choline-, ascorbic acid-, and para-aminobenzoic acid-deficient diets were similar to those in phase I except that 45 units of vitamin A palmitate were substituted for 1.2 milligrams of beta carotene, and menadione was increased from 4.0 to 8.0 milligrams per 100 grams of diet (dry weight), for reasons discussed later.

At the end of the 2-week acclimatization period preceding each feeding trial, 25 fish (7 to 12 grams each) were stocked into each of the 20 troughs. Lots were randomly selected to receive either the vitamin-complete diet or 1 of the 4 vitamin-deficient diets. Each diet was fed to 4 lots, a total of 100 fish.

The calculated amounts of wet food to be fed daily, 12 percent of the total weight of fish in each trough, were placed in marked containers and fed to the fish in 4 approximately equal portions. Feeding allowances were revised at 3-week intervals.

The fish were observed several times daily during the entire test period. Closer examinations were made during each weighing period, every third week. Gross post-mortem examinations were made of the livers, spleens, stomachs, intestines, and gill filaments of fish that died during the test period and of a number of the fish at the termination of the experiments. No histological examinations were made.

The need for a vitamin in the diet was indicated by differences in weight gain and mortality between channel catfish fed the vitamin-complete diet and those fed the vitamin-deficient diet, using an analysis of variance technique (Goulden, 1952, p. 71).

RESULTS

PHASE I

The following results were obtained from feeding the vitamin-complete and the pyridoxine-, pantothenic acid-, thiamine- and riboflavin-deficient test diets over a 24-week period.

1. Pyridoxine-deficient diet.--Results illustrated in figure 1 show no difference in the weight gain or mortality of the fish during the first 6 weeks; statistically significant differences in the weight gain occurred in 9 weeks. The variance ratio continued to increase, so that at the end of 15 weeks the difference in weight gain is significant at the 1-percent level. A significant difference in the mortality occurs at 12 weeks.

During the ninth week, nervous disorders developed among the fish fed the pyridoxine-deficient diet. Fish darted to the surface, swam in circles, whirled and twisted, and sometimes hit the sides of the troughs. These gyrations continued for 1 to 3 minutes; then the fish sank to the bottom, lay on their sides with mouths open, opercles extended, and the body

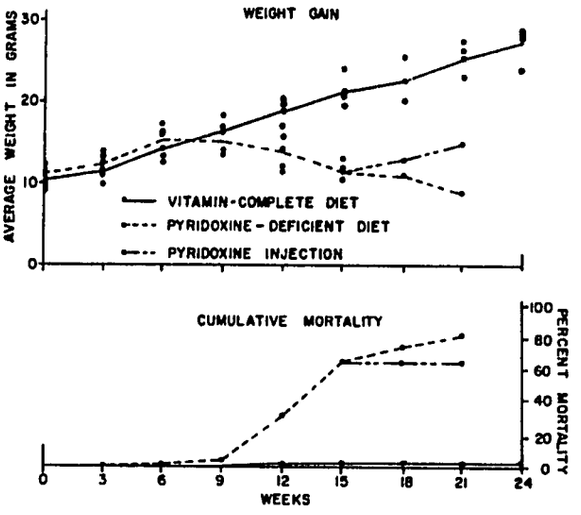

Figure 1.--Comparison of average weight gains and cumulative mortalities of four troughs of channel catfish fed pyridoxine-deficient diet and four troughs of channel catfish fed vitamin-complete diet.

in tetany. This condition continued for 2 to 5 minutes; then the fish usually righted themselves and swam away showing no ill effects. Fish had from one to five seizures before dying. On post-mortem examination, the liver, kidney, spleen, and gill filaments appeared normal. The size of the stomach and intestine was reduced, but this could have been due to their being empty.

At the end of 15 weeks, after definite vitamin-deficiency symptoms had been observed, the remaining fish on the pyridoxine-deficient diet were consolidated and then randomly divided into two groups of 19 fish each. One group was given a single injection of an aqueous solution of crystalline pyridoxine-HCl and then fed the vitamin-complete diet; the other group was not injected and was continued on the pyridoxine-deficient diet. The amount of pyridoxine-HCl injected (2.5 milligrams per kilogram body weight) was approximately twice the amount contained in a daily allowance of the vitamin-complete diet.

After an injection of pyridoxine-HCl, the previously pyridoxine-deficient group began to eat and to grow again, whereas the uninjected group did not begin to eat and continued to lose weight. However, there is no statistically significant difference in the weight gain between the two groups. Mortality ceased in the injected group but not in the uninjected group.

1. Pantothenic acid-deficient diet.--Results illustrated in figure 2 show no difference in the weight gain or mortality during the first 6 weeks; at the end of 9 weeks, the weight variance ratio approached statistical significance at the 5-percent level. By the end of 12 weeks a weight difference, significant at the 1-percent level, was accompanied by a significant difference in mortality.

The fish fed the pantothenic acid-deficient diet appeared normal for the first 6 weeks. Afterward food consumption declined, and by week 13 all feeding ceased. During the seventh and eighth weeks, the fish became less active. At the end of the eighth week several were lying on the bottom of the troughs and moved only when disturbed by other fish or by the investigator. These fish were the first to die and, as the experiment progressed, greater numbers of the fish demonstrated this symptom.

The gills of most of the fish that were living at the end of 12 weeks were covered with excessive mucous. Most of the gill filaments were

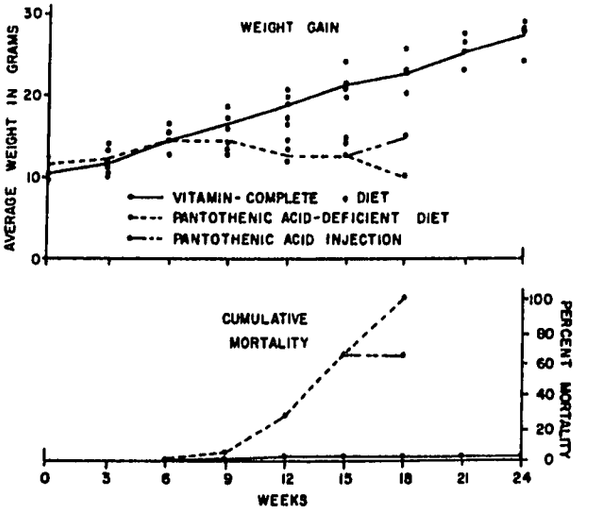

Figure 2.--Comparison of average weight gains and cumulative mortalities of four troughs of channel catfish fed pantothenic acid-deficient diet and four troughs of channel catfish fed vitamin-complete diet.

eroded or the lamellae were "clubbed". The opercles of many of the individuals were eroded. The gill membranes and lower jaw were absent in some fish. The fish in general had flabby body tissues, and the skin was white-grey in color. The fins of most fish were eroded to their bases, and the barbels were in various states of erosion, varying from erosion of the tip to complete absence. In the last 3 weeks of the experiment, small amounts of fungus were found on the fins and barbel stumps.

At the end of 15 weeks the remaining fish on the pantothenic acid-deficient diet were consolidated and randomly divided into two groups of 20 fish each. One group received a single injection of an aqueous solution of calcium pantothenate and then was fed the vitamin-complete diet; the other group was not injected and was continued on the pantothenic acid-deficient diet. The amount of calcium pantothenate injected (17 milligrams per kilogram body weight) was approximately twice the amount contained in a daily allowance of the vitamin-complete diet.

The injected fish began to eat and to grow again; the control group did not eat and continued to lose weight. There is no statistically significant difference in the weight gains of the two groups. Mortality ceased in the injected group but not among the uninjected fish.

3. Riboflavin-deficient diet.--Results illustrated in figure 3 show no significant statistical difference in weight gain or mortality between the fish fed deficient or complete diets during the first 18 weeks, but the variance ratios of the weight gains increased rapidly after 12 weeks. The difference in mortality of the groups for the 18-week period is highly significant. At 24 weeks the difference in weight gains is statistically significant at the 1 percent level.

The fish fed the riboflavin-deficient diet reacted normally for the first 15 weeks except for a slight reduction in food consumption after 11 weeks. Between weeks 15 and 22, food intake was nil; by week 24, normal food consumption was resumed, but the fish continued to lose weight.

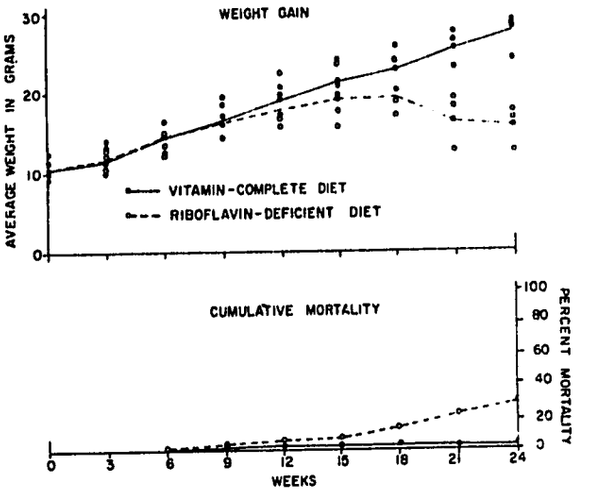

Figure 3.--Comparison of the average weight gains and cumulative mortalities of four troughs of channel catfish fed the riboflavin-deficient diet and four troughs of channel catfish fed the vitamin-complete diet.

Examination of the riboflavin-deficient fish revealed opaque lenses in one or both eyes of approximately 20 individuals. The gill filaments, livers, stomachs and intestines appeared normal.

4. Thiamine-deficient diet.--There appeared to be no difference in the weight gain during the first 9 weeks between fish fed deficient or control diets. Thereafter, the average weights showed an increasing divergence. At the end of 24 weeks, the variance ratio is statistically significant at the 1-percent level. There is no significant difference in cumulative mortalities between the two diet groups at the end of 24 weeks. These results are illustrated in figure 4.

The fish on the thiamine-deficient diet appeared normal for the first 18 weeks, but during weeks 19 and 20, food consumption decreased and continued to decline through week 24.

During the last weeks of the test, the fish fed the deficient diet were lethargic and had difficulty in maintaining equilibrium. Furthermore, it appeared that the most affected fish had difficulty in seeing and grasping and swallowing the food.

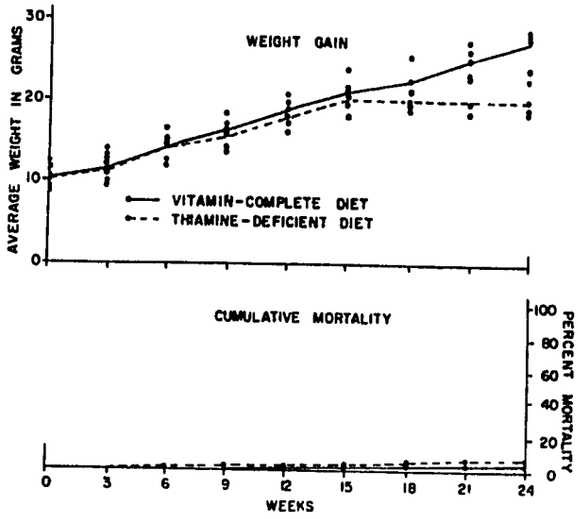

Figure 4.--Comparison of the average weight gains and cumulative mortalities of four troughs of channel catfish fed the thiamine-deficient diet and four troughs of channel catfish fed the vitamin-complete diet.

The stomach, liver, intestine, spleen, kidney, and gill filaments of the fish fed the vitamin-deficient diet appeared normal.

POST-PHASE I OBSERVATION

After the conclusion of phase I of the experiment and before the test fish were discarded to make room for phase II of the experiment, hemorrhagic areas were observed near the eyes of the fish fed the vitamin-complete and the thiamine- and riboflavin-deficient diets. Approximately 3 weeks later, hemorrhages were seen at the base of the fins, on the surface of the body, and in a few cases within the body cavity. Post-mortem examination revealed pale red livers, spleens, and gill filaments. The hemorrhagic condition (presumed to be caused by a deficiency of vitamin K--Murphy, 1939, and Schonheyder, 1935) was eliminated when the amount of menadione (synthetic vitamin K) was increased from 4 to 8 milligrams per 100 grams of food (dry weight).

During the last weeks of phase I of the experiment, slightly protruding eyes (pop-eyes) were observed in all groups of fish. The condition became progressively worse-- "pop-eyes" in all the fish and fluid-enlarged abdominal cavities in approximately 20 percent of the fish. The fluid was easily removed with a syringe, but the abdominal cavity usually refilled within a week. A visual examination revealed hemorrhagic kidneys and edema of the body cavity.

Since similar symptoms have been associated with vitamin A deficiencies in other animals (Wolbach and Howe, 1925) and since it has been reported that some animals cannot metabolize beta-carotene to vitamin A (Collins, Love, and Morton, 1935), 45 units of vitamin A palmitate were substituted for the 1.2 milligrams of beta-carotene in the diets. This level of vitamin A palmitate was suggested by the staff of the Poultry Department, Auburn University, Auburn, Ala. Within a period of 1 week, "pop-eye" was eliminated, and no new cases of fluid in the body cavity were observed. However, those fish already having a fluid-enlarged abdominal cavity did not recover. Possibly this "deficiency syndrome" was due either to an insufficient amount of beta-carotene in the diet or to the inability of channel catfish to metabolize sufficient amounts of beta-carotene to vitamin A to meet the vitamin A requirement. It is probably the latter, since 1.2 milligrams of beta-carotene is equivalent to approximately 2,000 units of vitamin A, but only 45 units of vitamin A per 100 grams of diet (dry weight) meet the needs of the fish.

PHASE II

The following results were obtained from feeding the vitamin-complete and the folic acid-, nicotinic acid-, biotin-, and inositol-deficient test diets over a 30-week period.

5. Folic acid-deficient diet.--The results are illustrated in figure 5. Because of variation within the four replicates, there is no significant statistical difference between the weight gains of the fish fed the two diets. The variance ratios increase with time, approaching significance at the 5 percent level by the end of 30 weeks. At this time, the average weight of the fish fed the vitamin-complete diet was 32 percent greater than the average weight of the fish fed the folic acid-deficient diet. The difference in cumulative mortality

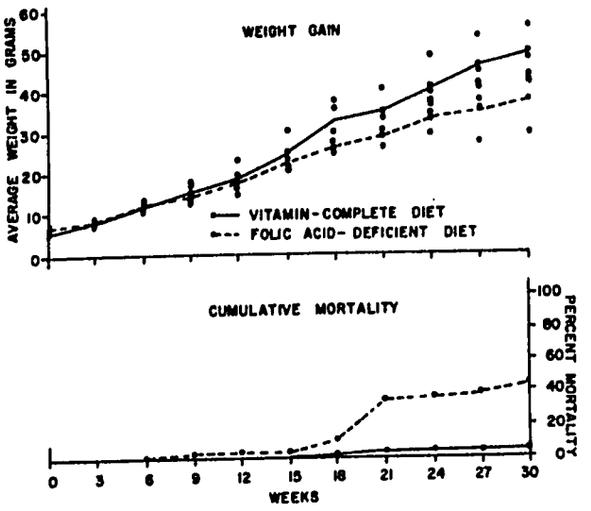

Figure 5.--Comparison of the average weight gains and the cumulative mortalities of four troughs of channel catfish fed the folic acid-deficient diet and four troughs of channel catfish fed the vitamin-complete diet.

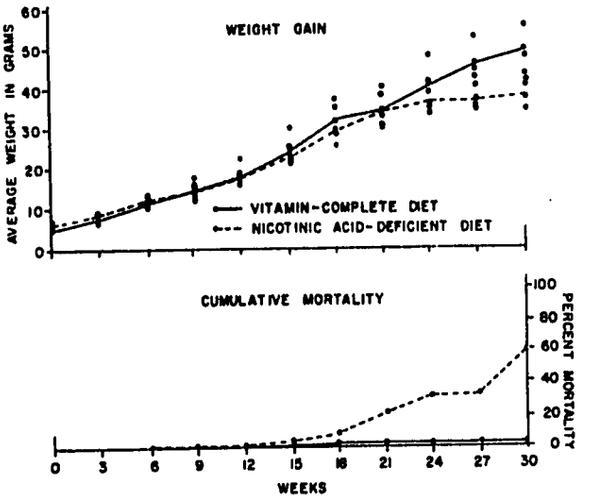

Figure 6.--Comparison of the average weight gains and the cumulative mortalities of four troughs of channel catfish fed the nicotinic acid-deficient diet and four troughs of channel catfish fed the vitamin-complete diet.

between the folic acid-deficient group and the vitamin-complete group at the end of 21 weeks is highly significant.

The fish fed the deficiency diet appeared normal for the first 15 weeks, except for a slight decrease in food consumption. During the next 3 weeks, there was extreme reduction in the amount of food eaten, accompanied by decreased activity and weight gain.

Gross post-mortem examinations revealed normal internal organs, except for slightly darkened spleens and livers.

6. Nicotinic acid-deficient diet.--The results are illustrated in figure 6. Variation within the replicates precluded significant statistical difference in weight gain between fish fed the deficiency and control diets. However, the variance ratios increase with time, approaching significance at the 5 percent level by week 30. At the end of the experimental period the average weight of the fish fed the complete diet was 28 percent greater than that of fish fed the nocotinic acid-deficient diet.

The difference in the cumulative mortalities of the nicotinic acid-deficient group and the vitamin-complete group is highly significant at the end of 24 weeks. At the end of 30 weeks, the mortality of the group fed the nicotinic acid-deficient diet was 10 times that of the group fed the vitamin-complete diet.

The fish fed the nicotinic acid-deficient diet appeared normal for the first 15 weeks with the exception of a slight decrease in the amount of food eaten and the death of three fish. The death of the three fish occurred immediately after weighing, and I assumed that the fish might have been injured in the weighing process. However, all subsequent mortalities also occurred during or soon after the periodic weighings.

Presumably, these deaths were due to stress susceptibility induced by the nicotinic acid deficiency, since such "handling mortality" was not observed in the vitamin complete group or the other vitamin deficient groups. The affected fish when returned to the trough would immediately sink to the bottom and lie on their sides. Their bodies were rigid; the opercles were spread, but little or no opercular movement took place. A number of fish exhibited lethargy and reduced coordination prior to death which usually occurred within 1 to 6 hours after the weighing.

Kidneys, livers, stomachs, spleens, and intestines appeared normal.

7. Biotin-deficient diet.--Comparative results of feeding biotin-deficient and vitamin-complete diets are illustrated in figure 7.

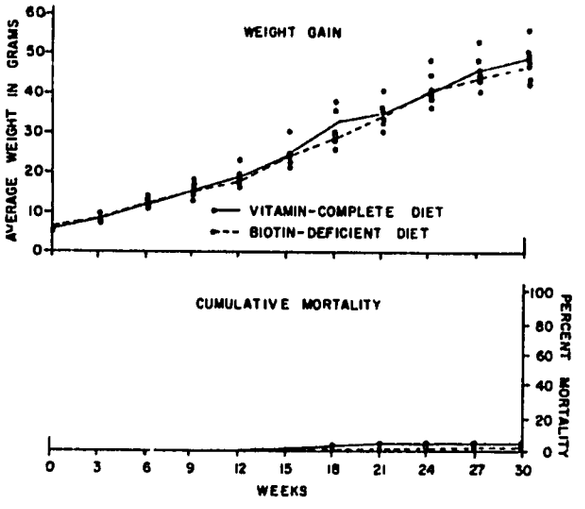

Figure 7.--Comparison of the average weight gains and cumulative mortalities of four troughs of channel catfish fed the biotin-deficient diet and four troughs of channel catfish fed the vitamin-complete diet.

There is no difference in weight gain or mortality. Postperiod inspection of 10 fish revealed no abnormalities of gill filaments, stomachs, livers, spleens, intestines, or kidneys.

It should not be assumed that channel catfish do not require biotin. Although no need was demonstrated during the 30-week experimental period, this vitamin is required by man and most animals, and it may well be essential in the diet of channel catfish.

8. Inositol-deficient diet.--The results of feeding the inositol-deficient and vitamin-complete diets are illustrated in figure 8. No differences in weight gain or mortality were demonstrated, nor did postperiod examination of 10 fish show any abnormal conditions in observed organs.

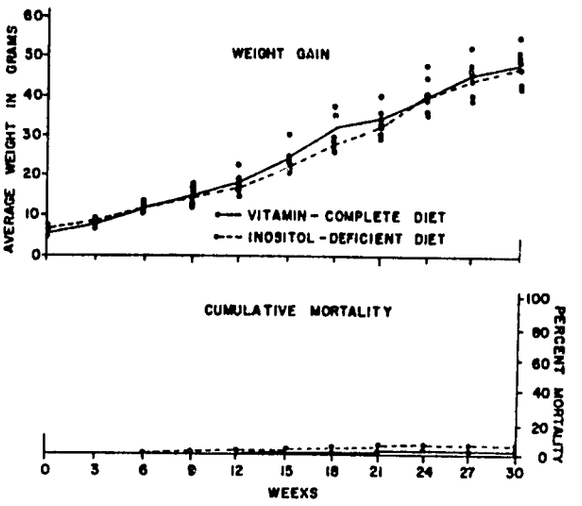

Figure 8.--Comparison of the average weight gains and cumulative mortalities of four troughs of channel catfish fed the inositol-deficient diet and four troughs of channel catfish fed the vitamin-complete diet.

PHASE III

The following were obtained from feeding the vitamin-complete and the B-12-, choline-, ascorbic acid- and para-aminobenzoic acid-deficient diets for a 36-week period.

9. Vitamin B-12-deficient diet.--Figure 9 illustrates the results of the experiment. For the first 21 weeks there is no significant statistical difference in weight gain between the fish fed the two diets; by week 36, the difference in weight gain is significant at the 1-percent level. The average weight of the fish fed the vitamin-complete diet was 39 percent greater than the average weight of the fish fed the B-12-deficient diet. There is no significant difference in the mortality between the groups. There was no mortality in the vitamin-deficient group, but three control fish were lost, probably because of injury during weighing.

Fish fed the vitamin-B-12-deficient diet appeared normal for the first 21 weeks; after week 24, food consumption was slightly reduced. No abnormal conditions of the gill filaments, livers, spleens, kidneys, or intestines

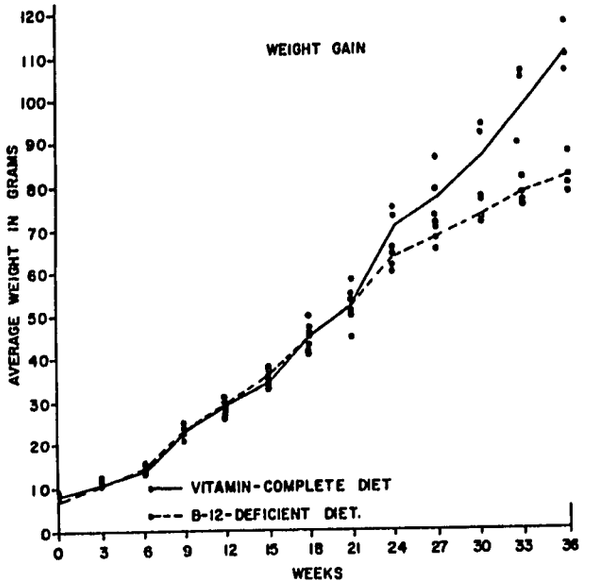

Figure 9.--Comparison of the average weight gains of four troughs of channel catfish fed the vitamin-B-12-deficient diet and four troughs of channel catfish fed the vitamin-complete diet. At the end of 36 weeks, cumulative mortalities for the vitamin-B-12-deficient group and the control group were 0 and 3 percent, respectively.

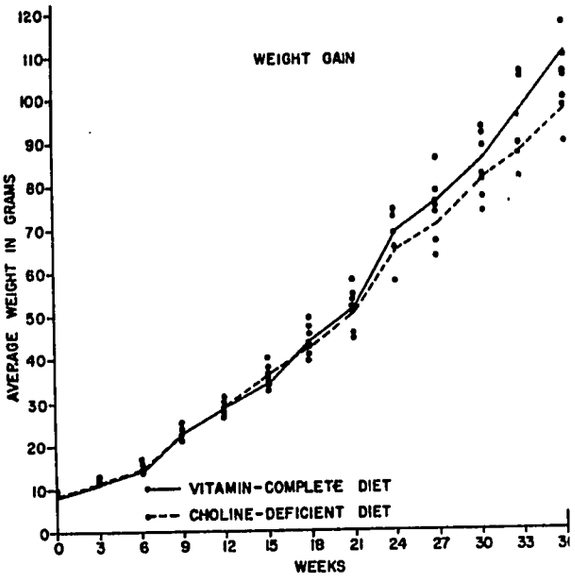

Figure 10.--Comparison of the average weight gains of four troughs of channel catfish fed the choline-deficient diet and four troughs of channel catfish fed the vitamin-complete diet. At the end of 36 weeks the cumulative mortalities for the choline-deficient group and the control group were 2 and 3 percent, respectively.

were observed during the postperiod examination of 10 fish.

10. Choline-deficient diet.--The consequences of the choline deficiency diet are illustrated in figure 10. There is no significant statistical difference in the weight gain of the fish during the first 21 weeks; by week 24 the difference in weight gain is significant at the 5-percent level, and by week 33 the variance ratio is significant at the 1-percent level. It should be noted that these fish on choline-free diet show substantial weight gain. Most animals show a greater growth dependence on choline than any of the other vitamins since choline is needed for building phospolipids that are essential for growth as well as having other classical vitamin functions. There is no significant difference in the mortality of the two groups of fish. Cumulative mortality at the end of 36 weeks for the choline-deficient group was 2 percent and for the control group was 3 percent.

Two fish died during week 33. Post-mortem examination revealed enlarged livers and hemorrhagic areas in the kidneys. The stomachs, spleens, and gill filaments appeared normal. In postperiod examination of 10 fish, 3 weeks later, moderate to greatly enlarged livers wer found. Hemorrhagic areas in the kidneys were observed, but other organs and tissues appeared normal.

11. Ascorbic acid-deficient diet.--The results are illustrated in figure 11. There is no difference in the weight gain or mortality of the fish fed deficient and control diets. At the end of 36 weeks cumulative mortality in the ascorbic acid-deficient group was 2 percent and in the control group was 3 percent. Postperiod examination of 10 fish exposed no abnormal conditions of the gill filaments, stomachs, spleens, intestines, or kidneys.

12. Para-aminobenzoic acid-deficient diet.--The results are illustrated in figure 12. There is no significant statistical difference in the weight gain or mortality between the compared groups of fish. Cumulative mortality for the para-aminobenzoic acid-deficient group was 1 percent and for the control group was 3 percent.

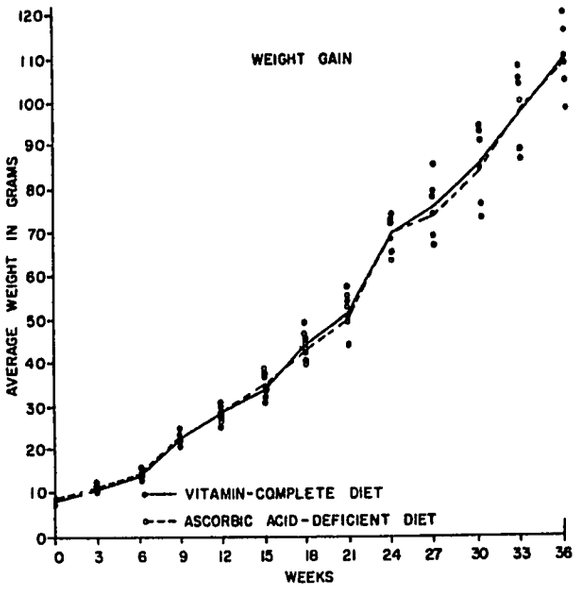

Figure 11.--Comparison of the average weight gains of four troughs of channel catfish fed the ascorbic acid-deficient diet and four troughs of channel catfish fed the vitamin-complete diet. At the end of 36 weeks, cumulative mortality was 2 percent in the ascorbic acid-deficient group and 3 percent in the control group.

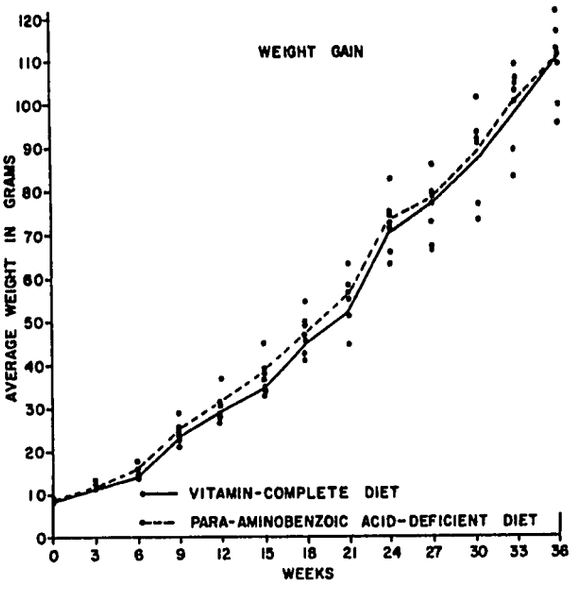

Figure 12.--Comparison of the average weight gains of four troughs of channel catfish fed the para-aminobenzoic acid-deficient diet and four troughs of channel catfish fed the vitamin-complete diet. At the end of 36 weeks, cumulative mortality was 1 percent in the para-aminobenzoic acid-deficient group and 3 percent in the control group.

At the termination of the experiment, 10 fish were examined. No abnormal conditions were noted in the gill filaments, stomachs, livers, spleens, intestines, or kidneys.

CONCLUSIONS AND SUMMARY

Vitamin-deficiency symptoms were identified in channel catfish that were fed diets deficient in the water-soluble vitamins pyridoxine, pantothenic acid, riboflavin, thiamine, folic acid, nicotinic acid, B-12, or choline. Fat-soluble vitamin A and vitamin K deficiency symptoms were observed after feeding diets that contained beta-carotene or 4.0 milligrams of menadione (synthetic vitamin K) per 100 grams of food (dry weight). With subsequent diets these deficiencies were not evident after substituting vitamin A palmitate for beta-carotene and doubling the content of menadione. No dietary need was observed for the water-soluble vitamins biotin, inositol, ascorbic acid, and para-aminobenzoic acid. It should be pointed out that some of these vitamins are essential for trout and other animals and may be essential in the diet of channel catfish. The need for the fat-soluble vitamin D and E are not tested.

The effects of feeding vitamin-deficient diets to channel catfish are tabulated as follows:

Vitamin deficiency	Effects
1. Pyridoxine.....	Erratic swimming, tetany, gyrations and muscular spasms when stressed, reduced weight gain and mortality.
2. Pantothenic acid.....	"Flabby" body tissues, "mummy" textured skin, excessive mucous on gills, clubbed gill filaments, and eroded gill membranes, lower jaw, fins and barbels, lethargy, reduced weight gain and mortality.
3. Riboflavin.....	Opaque lens of one or both eyes, mortality.

Vitamin deficiency	Effects
4. Thiamine	Reduced weight gain, lethargy, and difficulty in maintaining equilibrium.
5. Folic acid	Lethargy, reduced food consumption, mortality.
6. Nicotinic acid	Tetany and eventual death brought about by stress, lethargy, reduced coordination. (Author's note: When this experiment was repeated in 1961, it was observed that the fish resided in the darkened areas of the aquarium and would venture into well-lighted areas only to feed or when disturbed.)
7. B-12	Reduced weight gain.
8. Choline	Hemorrhagic areas in the kidneys and enlarged livers; reduced weight gain.
9. A	"Pop-eye"; fluid in body cavity, hemorrhagic kidneys, and edema of the body cavity.
10. K	Hemorrhages on body surface.

AUTHOR'S NOTE

In 1961, this experiment was repeated at the Southeastern Fish Cultural Laboratory, Marion, Ala. The diets were agar-bound and contained 33 percent protein. The results were essentially the same as those reported in this paper except that weight gain was faster and the deficiency symptoms developed within one-third to one half the time of the previous experiment. It is noteworthy that the deficiency symptoms were observed after approximately the same percent of weight gain in both experiments.

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