RAT-FEEDING STUDIES TO DETERMINE PRESENCE OF ANTIMETABOLITES, WATER-SOLUBLE VITAMINS, AND ESSENTIAL MINERALS IN RAW MENHADEN AS COMPARED WITH RAW HADDOCK AND BEEF

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ABSTRACT

A rat-feeding study was conducted to determine the possible presence of antimetabolites other than thiaminase in raw menhaden and to evaluate the contribution of the watersoluble vitamins and minerals in menhaden in meeting an animal's requirements for growth. For comparison, these factors were evaluated in raw haddock fillets, which do not contain thiaminase, and in raw beef round. It was found that the raw menhaden contained no antimetabolites affecting growth, other than thiaminase, and contributed considerably toward meeting an animal's requirements for essential minerals and for the water-soluble vitamins other than thiamine. Haddock fillets contained no antimetabolites, contributed a lower level of vitamins for growth than did menhaden, but apparently contained a higher level or a better balance of essential minerals. Beef round probably contained no antimetabolite, contributed about the same level of vitamins for growth as did menhaden, but apparently contained a lower level or poorer balance of essential minerals than did either menhaden or haddock.

INTRODUCTION

It is known that certain raw fishery products contain the enzyme thiaminase, which destroys thiamine (Lee 1948; Yudkin, 1949). This fact is important to fur farmers, who feed large quantities of raw fish to their fox and mink. Special precautions must be taken to avoic the effects of this antimetabolite when thiaminase-containing fish are included in the diet, or

a thiamine deficiency disease, commonly called Chastek paralysis, may develop. Fur farmers often alternate the feeding of fish containing thiaminase with fish lacking thiaminase or with other protein food. Thiaminase can be destroyed by cooking the fish, since enzymes are heat labile. Nevertheless, many fur farmers prefer to feed the fish raw.

Although a regimen of alternate daily feeding of thiaminase-containing fish with other high-quality protein foods is apparently successful, many farmers and other nutritionists surmise that these fish may contain additional antiwater-soluble-vitamin factors. If this speculation were found to be true, further precautions during feeding would be necessary.

Also, although it is known that thiaminase-containing whole raw fish will contribute no thiamine to the diet, little is



Fig. 1 - Staff member holding black-hooded rat shows equipment used in the antimetabolite study.

known regarding the possible contributions of these fish in meeting an animal's requirements of other water-soluble vitamins and of necessary minerals. Such information would aid in

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U. S. DEPARTMENT OF THE INTERIOR FISH AND WILDLIFE SERVICE SEP. NO. 648 the efficient and economical formulation of animal diets with both thiaminase and nonthiaminase-containing fish.

Raw, whole menhaden, which contains thiaminase, represents a food source of great potential value to the fur farmer. This industrial species of fish is quite abundant and should be available to the fur farmer at a low cost compared to other fish and land-animal meat. Menhaden apparently, in limited trials, has been successfully fed to mink under controlled conditions (Anonymous 1960).

The object of the study reported here, therefore, was to conduct animal-feeding studies to determine the possible presence of other antimetabolites in menhaden and to evaluate the contribution of this species in meeting animal requirements for water-soluble vitamins and nutritionally-important minerals. Rats were utilized in the study because of unavailability of mink at this laboratory. The fillets of raw haddock and raw round of beef, fish and landanimal meat supposedly containing no antimetabolites, were included for testing and represent extremes of food sources to serve as comparisons and controls against the menhaden.

EXPERIMENTAL PROCEDURE

Groups of weaned rats of a highly inbred, black-hooded strain were fed the various diets summarized in table 1. The rats had free access to water. The high-energy supplement and the meat or fish were placed in separate feeding cups for free-choice selection.

Diet Designation	Dietary Components					
	High Energy Formulation ¹ /	Whole Menhaden ² /	Haddock Fillets2/	Beef Round2/	Thiamine Supplementation Only <u>3</u> /	Complete Water- Soluble Vitamin Supplementation4
1 .	X	X		-		
2	X	X	-	-	X	- 11
3	X	X	-	-	-	X
4	X	-	Х	-	-	-
5	X	-	Х		X	-
6	X	-	X	-	-	X
7	X	-	-	X	-	
8	X	-	-	X	X	-
9	X	-	-	X	_	X

1/Consists of sucrose, lard, cod-liver oil (in the proportions of 80, 16, and 4 parts by weight, respectively) and 0.02 grams vitamin E per 2,000 grams of diet--as a source of carbohydrate, fat, and fat-soluble vitamins.

2/Raw and carefully ground to avoid altering any enzymes-as a source of protein, water-soluble vitamins, minerals, and energy.
3/Oral daily supplementation of approximately 10 times the daily requirement fed with tuberculine syringe (0.2 milliliters of a thiamine hydrochloride solution of 10 milliliters thiamine HCl in 100 milliliters of water per 50 grams body weight).
4/Oral supplementation of thiamine as above plus approximately two and one-half times daily requirement of each of the water-soluble vitamins fed in the drinking water.

Menhaden were obtained in excellent condition from a boat that had been at sea off the coast of North Carolina two days. The menhaden was frozen immediately after the boat was docked. At the laboratory, the frozen fish were ground in a Hobart meat grinder and placed in plastic bags for storage. Haddock fillets and beef round were purchased at a local supermarket. The packaged haddock fillets were prefrozen and were not ground for feeding purposes. The fillets were stored in the original containers. The beef round was carefully trimmed of excess fat, ground with a Hobart grinder, and then frozen. All of these foodstuffs were held at 0° F. until shortly before the daily feedings.

Thiamine, when fed, was administered orally (table 1) to each rat rather than placed in the drinking water. (Data obtained from initial studies indicate that thiamine held in the drinking water is destroyed.)

Four rats, two males and two females, weighing 50 to 56 grams, were randomly allotted to each group. Not more than one litter-mate was allotted to any single group. The rats were housed individually in cages fitted on wire screens. The temperature of the room was maintained at 80° $\pm 2^{\circ}$ F., and the humidity was maintained at 65 ± 5 percent. Daily records were kept of weights during the 4-week study, and observations were recorded of any notice-able physiological changes in the animals.

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SUMMARY OF RESULTS AND DISCUSSION

The data are presented in figure 2.



Fig. 2 - Gain in weight of groups of rats fed diets containing high-energy supplementation and fish or meat with and without vitamin supplementation.

MENHADEN DIET: The group of rats fed the diet containing menhaden and no vitamin supplementation (diet 1) began to lose weight on the 12th day and continued to lose weight thereafter. Characteristic symptoms of avitaminosis were noted during the testing period. These symptoms included loss of appetite during the second week, followed by loss of weight and loss of muscular coordination, capillary fragility, and sensitivity to touch during the third and fourth weeks of the study. By the fourth week, a marked difference in weight and physiological condition was noted for these rats compared to those receiving the same diet but with thiamine supplementation (diet 2) and with complete water-soluble vitamin supplementation (diet 3). Rats gained weight slowly when fed menhaden with thiamine supplementation (diet 2) but did not exhibit the deficiency symptoms observed with the rats of group 1. Rats fed menhaden and all the vitamins (diet 3) gained considerably more weight than did the rats fed diets 1 and 2. The mean gain of the rats fed diet 3 was about 80 percent of that normally obtained when rats of this colony are fed diets well balanced in nutrients.

These results indicate that raw whole menhaden (1) is thiamine deficient, as was expected, since menhaden contains thiaminase; (2) does not contain other antimetabolites detrimental to growth of rats; (3) contains sufficient levels of water-soluble vitamins (other than thiamine) to permit growth; and (4) contains sufficient levels of essential minerals to permit good growth. Results also indicate that thiaminase is the primary limiting factor for growth of rats under the feeding conditions described.

HADDOCK DIET: Rats fed the diet containing haddock (diet 4) gained well during the first week. Periods of loss of weight were observed during the second week. The rats lost weight slowly during the last 2 weeks and more noticeably during the last few days of the study. When fed the same diet with thiamine supplementation (diet 5), rats gained weight slowly until the last 2 days when they lost weight. When all the vitamins were added to the diet (diet 6), the rats gained considerable weight-nearly the optimum expected with rats of this colony. This gain was markedly greater than that obtained when the rats were fed the diet containing haddock with no vitamins (diet 4) or haddock with thiamine supplementation (diet 5).

These results indicate that raw haddock fillets (1) do not contain thiaminase or any other antimetabolite detrimental to growth, (2) contain a level of water-soluble vitamins barely adequate to maintain weight, and (3) contain levels of essential minerals to permit nearly optimum growth. Results also indicate that one or more water-soluble vitamins is the limiting factor for the support of growth of rats under the feeding conditions described. It was observed that some edema and tenderness developed in the rats during the last week of the study. The reason for this condition is not clear. The edema did not seem to be sufficiently acute to account for much of the gain in weight of the animals.

<u>BEEF DIET</u>: Rats fed diets containing beef with no vitamins and with thiamine supplementation (diets 7 and 8) gained fairly well until about the 13th day and then gained slowly until the last week of the study. These groups lost weight noticeably during the last 4 days of the study. The final weight of these rats was greater than the weight of the rats fed the menhaden with no vitamins (diet 1), and the rats gained weight somewhat better than did those fed diets 4 and 5. Even so, they lost weight during the last 5 days of the study, as did the rats fed diets 4 and 5. The final weight of the rats fed beef and a complete vitamin supplementation (diet 9) was not as great as rats fed either menhaden or haddock with a complete vitamin supplementation (diets 3 and 6), although the levels and ratios of high-energy supplement and meat or fish consumed by the rats fed these diets were similiar. Before the loss of weight, the growth of the group fed diet 9 was about equal to that of the group fed diet 3 but considerably less than that of the group fed diet 6.

These results indicate that raw beef round (1) probably does not contain thiaminase or any other antimetabolite detrimental to growth and (2) contains a level of water-soluble vitamins sufficient to permit fair growth for a limited time. The results also indicate that one or more of the water-soluble vitamins is primarily a limiting factor for support of growth and that there may be an additional limiting factor for growth of rats, not definable by this study, that is present in beef and is not present in fish. Quite possibly, this additional limiting factor for growth in beef may be that the minerals necessary for growth are not present in adequate amounts in beef and are present in adequate amounts in fish.

EFFECT ON HAIR COLOR: It was observed near the end of the study that the normally dark brown portion of the hair of the rats fed diets 4, 5, 6, 7, 8, and 9 turned silvery grey. The reason for this change in hair color is not apparent.

CONCLUSIONS

Results indicate that raw whole menhaden contains no antimetabolites 1/ other than thiaminase and that they contribute considerably toward meeting an animal's requirements for minerals and for the water-soluble vitamins other than thiamine. Raw haddock fillets contain no antimetabolites, contribute a lower level of vitamins for growth than does menhaden. but apparently a higher level of essential minerals. Raw beef round probably contains no antimetabolites, contributes about the same level of vitamins for growth as menhaden, but apparently a lower level of essential minerals than either menhaden or haddock.

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1/For the purposes of this report, antimetabolites include only antiwater-soluble-vitamin factors.



HERRING ROE INDUSTRY GETS START IN NORWAY

Herring roe, specially salted, is a new export product of Norway. In Japan, known as "Kazanoko," it is an expensive delicacy eaten in big quantities during the New Year celebrations. Last year was the first time a shipment of 220 pounds was sent to Japan. This year, between 20 and 30 fish plants in Norway are making "Kazanoko."

"Kazanoko" is an old tradition in Japan. Among other things, it is supposed to increase fertility. The name, "Kazanoko" translated directly means "more sons and daughters."

The salted roe is eaten without any special preparation. It is eaten especially with rice wine. Since Japan lost most of her herring production territory, she has turned to Norway for this special delicacy. But the 1961 herring failure in Norway means that there won't be much herring roe this year.

The herring roe which is mostly used is "Moree Coast Eel, glass-rogn." It is an over-ripe herring roe, soft like jelly, which used to be thrown away. The roe is salted for three days to make it hard and solid. After three days, it is washed and then salted again. Then it is also frozen. (The Fisherman, April 14, 1961.)

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