DEVELOPMENT OF METHODS FOR BIOASSAY OF GROWTH FACTORS PRESENT IN FISHERY INDUSTRIAL PRODUCTS--PRELIMINARY INVESTIGATION OF PRESENCE OF UNIDENTIFIED NUTRITIONAL FACTORS[1/]

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ABSTRACT

PRELIMINARY STUDIES OF PHYSIOLOGICAL STRESS-FACTORS IN TEST ANIMALS AS POSSIBLE METHODS OF MEASURING UNIDENTIFIED GROWTH-FACTORS PRESENT IN FISH MEALS WERE MADE. STUDIES WERE DESIGNED TO ELIMINATE GROWTH-MEASUREMENT AND THE MANY VARIABLES AFFECTING SUCH GROWTH, AS A METHOD OF MEASURING THE PRESENCE OF GROWTH FACTORS. ALPHA ESTRADIOL AND THYROACTIVE SUBSTANCES IN EXCESS WERE TESTED. A STUDY OF THE RESULTS SHOWED THIS BIOASSAY METHOD TO BE RELATIVELY EXPENSIVE AND TIME-CONSUMING. NO FURTHER STUDIES ARE NOW PLANNED.

Available data indicate that fish meal and condensed fish solubles contain, in addition to the known nutrients, factors essential for optimal growth and development of poultry and other animals. The data published in the literature, however, are equivocal because the effect of these "unidentified factors" could not be demonstrated in all cases under laboratory conditions. These diverse findings may be due to a number of reasons: (1) different batches of fish meal or fish solubles may contain different amounts of the active factor (or factors); (2) noxious or deleterious substances may have been produced in the preparation or processing of some of these supplements which masked the effect of the "unidentified growth factor;" (3) strain and species differences may exist in the animals' requirements for this factor(s); or (4) the growth-promoting effect of the unidentified factor in fish meal or condensed fish solubles may only be demonstrable in animals with certain kinds of intestinal flora. These complications not only retard work leading toward the isolation of the above unidentified factor(s) but make it difficult to determine the growth-promoting value of commercial fishery products.

The experiments undertaken in the present project had the objective of developing a practical and reliable method for bioassay of "unidentified nutritional factors" in fishery industrial products. It was recognized from the start that factors which might be shown to exist by such procedures may or may not be identical to the factor(s) in fishery industrial products which promotes growth. It was felt by the present investigators, however, that assay procedures might be developed which are a more sensitive index for the quantitative measurement of such factors than increment in body weight. Subsequent tests would have to establish the degree of correlation between the chick-growth effect and the results of the new assay procedures.

For a number of reasons it was felt that some index other than increment in body weight of the chick would be desirable as a bioassay procedure. Increment in body weight in the final analysis is the resultant of an almost endless number of factors. Genetic background, pretest diet, environmental temperature, nature and type of bacterial flora, degree of crowding, degree and type of infestation, physical state of the diet, particle size, moisture content of diet, taste of the ration, possible presence of noxious materials in the diet which might inhibit food intake--any one or more of the above as well as countless other factors might all serve to decrease food consumption and weight increment in the chick. Although it is recognized that whereas, from a practical point of view, a more rapid growth increment or a more efficient degree of food utilization is the objective of the poultryman, from the stand-

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point of a bioassay procedure, methods which are not so sensitive to the effects of slight alterations in food intake or some of the other factors indicated before might be more desirable.

From a historical point of view, virtually all essential nutrients were at one time or other "unidentified growth factors." In fact the existence of a new nutrient in many cases first became evident by feeding animals diets containing all nutrients known as of that time and then showing that the growth increment obtained on these diets could be increased by feeding a particular food or fraction thereof. If the active factor could subsequently be shown to be distinct from any of the known identified nutrients, the data might be interpreted as evidence for the existence of a new nutrient. In the early work on the concentration and isolation of such factors, these nutrients were assayed by their capacity to promote growth on rations deficient in these factors. It was only when further data became available as to the physiologic role of the various nutrients that other bioassay procedures could be devised that more specifically measured the particular nutrient in question (for example, odontoblast assay for vitamin C; blood levels of pyruvic acid in a thiamine assay; xanthuronic acid excretion as an assay procedure for vitamin B_6, etc.) than the increased growth. It is possible that a similar situation prevails in respect to an unidentified factor(s) in fishery industrial products. If an essential nutrient distinct from any of the known nutritional factors does exist in such material, it is possible that such a factor may exert demonstrable physiologic effects other than the indirect manifestations of increased growth. Experiments were accordingly undertaken in an effort to find such effects.

Available data indicate that in addition to the known nutrients substances are present in natural foodstuffs which are required in increased amounts under various conditions of stress. Such factors are apparently dispensable under normal conditions, or their requirements are so small they may readily be met by amounts present in the diet or through the synthetic activity of the intestinal flora or the animals' own tissues. Certain stressor agents or drugs, however, may increase requirements for these substances to such an extent that deficiencies occur, manifested by retarded growth or tissue pathology and preventable by the administration in appropriate amounts of the missing nutrient.

The induction of nutritional deficiencies by exposing animals to various stressor agents and the correction of these deficiencies by the administration of graded levels of the missing nutrient have been employed by a number of investigators as a bioassay procedure. This technique has proved of particular value in the bioassay of vitamin B12. Experiments undertaken in the present project have attempted to apply this technique to the development of a bioassay for unidentified physiologically active factors in fish meal and fish solubles. Evidence for the existence of such a factor(s) was obtained.

Available data indicate that the ovaries of immature rats fed a purified ration containing 10 milligrams of alpha-estradiol per kilogram of diet remain immature both in weight and microscopic appearance. This effect can be largely counteracted by the concurrent feeding of desiccated whole liver (Ershoff and McWilliams (1948) or dried alfalfa (Ershoff, Hernandez, and Mathews 1956). Experiments were undertaken to determine the effects of fish meal and fish solubles under comparable experimental conditions. Findings indicate that the deleterious effects of alpha-estradiol feeding on ovarian development in the immature rat could be largely counteracted by the concurrent feeding of sardine solbules or tuna solubles at a 5-percent level in the ration. Blended tuna meal when added at a 5-percent level in the diet was similarly active; sardine meal at a 5-percent level, however, was without activity. Samples of menhaden meal and menhaden solubles when fed at a 5-percent level in the diet were both inactive. The protective factor(s) was apparently distinct from any of the known nutrients. Supplements of all the known vitamins (both fat-soluble and water-soluble), salt mixture, corn oil, casein or cellulose, either alone or in combi-

nation, were without protective effect. Present findings suggest the possibility of employing reversal of alpha-estradiol toxicity as a bioassay procedure for an unidentified factor(s) in fishery industrial products. The method, however, has the disadvantage of being time-consuming and relatively expensive.

Available data indicate that rats fail to survive when fed purified diets containing massive doses of desiccated thyroid or thyroactive substances. Experiments were conducted which indicate that fish meal contains a factor(s) apparently distinct from any of the known nutrients which significantly prolonged the average survival time of hyperthyroid rats.

Findings indicate that both tuna meal and sardine meal when fed at a 5-percent level in the diet resulted in a significant increase in the average survival time of hyperthyroid rats fed a purified ration under conditions of the present experiment. Tuna solubles or sardine solubles had little if any protective effect. Supplementing the basal ration with protamone diet with additional amounts of the known vitamins, salt mixture roughage, 5-percent casein or corn oil at levels of 2-percent, 5-percent, or 10-percent of the diet was without beneficial effect. It would appear from these findings that tuna meal and sardine meal contain a factor or factors apparently distinct from any of the known nutrients which significantly prolonged the survival time of hyperthyroid rats fed a purified ration containing sucrose as the source of dietary carbohydrate and methionine-supplemented soy protein as the source of dietary protein. Findings suggest the possibility of employing increase in the average survival time of thyrotoxic rate as a bioassay procedure for an unidentified factor(s) in fish meal. The method, however, has the disadvantage of being time-consuming and relatively expensive.

Additional studies were undertaken with rats fed diets similar to the above but with casein replacing the methionine-supplemented soy protein as the source of dietary protein and with desiccated thyroid fed at a 0.5-percent level in place of the iodinate casein. On the latter diet, in contrast to the soy protein-containing ration, supplements of tuna meal or sardine meal had little if any beneficial effect as judged by increased length of survival. These findings suggest that the source of dietary protein may significantly affect requirements for the unidentified factor(s) present in fish meal.

LITERATURE CITED

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EAR BONES (OTOLITHS) USED TO DETERMINE AGE OF OCEAN PERCH

The age of ocean perch (Sebastes marinus) has been determined accurately from the ear bones, according to the Fish and Wildlife Service's Fishery Biologists at Woods Hole (Mass.) Laboratory. By making collections of ear bones from the same stock every three or four months it was established that a single ring was formed each year and that older specimens grew about 1 centimeter (0.39 inch) per year.