

ANIMAL PROTEIN FACTOR IN CONDENSED FISH SOLUBLES

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The existence of an unidentified animal protein factor in nutrition was first recognized by practical poultrymen, whose wartime all-plant rations failed to nourish growing chicks. Supplements such as meat scraps, liver meal and fish meal were found to correct the deficiency.

Condensed fish solubles (1) appeared to be an especially rich source of the factor, but exhaustive chemical analysis of this product (2) failed to explain its biological activity in terms of known essential nutrients. Recent work (3, 4, 5, 6, 7) has shown that for optimum growth the weanling albino rat likewise requires an animal protein factor found in condensed fish solubles (Fig. 1).

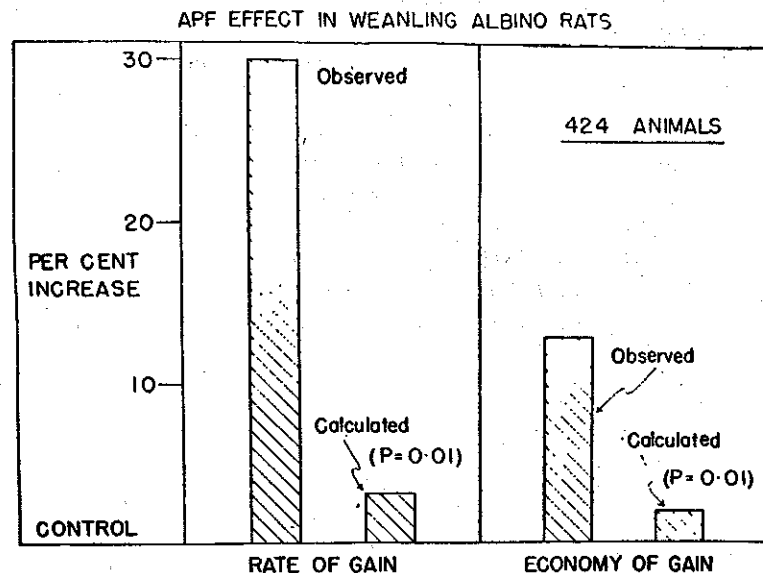


Fig. 1. APF effect in weanling albino rats. The base line represents response of control group (212), "observed" column height the increment of supplemented group (212). "Calculated" column height is derived from the data by Fisher's "t" method. Adapted from King and Hauge (5).

Use of the albino rat as a pilot animal has made possible studies with highly purified diets based upon isolated soybean protein (Table I).

TABLE I*
PURIFIED DIET FOR APF RAT TEST

INGREDIENT	per cent	VITAMIN	Micrograms per rat per day
Dextrose	65.0	Thiamine hydrochloride	90
Soy protein	20.0	Riboflavin	120
Distillery solubles	5.0	Niacin	500
Salt mixture	4.0	Calcium pantothenate	200
Cottonseed oil	3.5	Pyridoxine hydrochloride	200
Fiber	2.0	Choline chloride	20,000
A-D oil	0.5	Inositol	4,000
	100.0	p-Aminobenzoic acid	700
		Biotin	4
		Folic acid	4
		Menadione	50

*King and Hauge (3).

A fish solubles concentrate has shown growth-promoting activity in both rat (3) and chick (8). It is thus possible to evaluate animal protein factor activity (APF) by a simple and inexpensive rat growth test (Fig. 2).

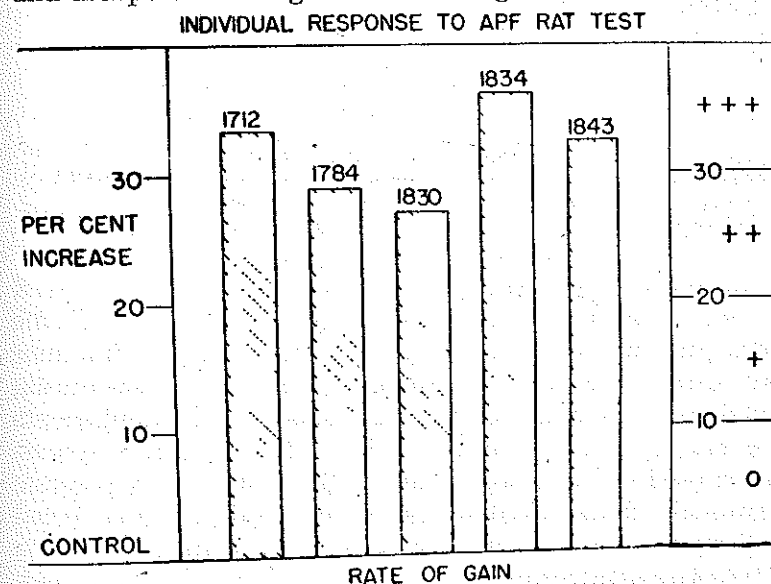


Fig. 2. Individual response to APF rat test. Increments are plotted as in Fig. 1, except that each column in Fig. 2 represents the increased response of a single test rat compared with its littermate control. Adapted from King and Hauge (4).

Two major types of condensed fish solubles are commercially available, representing Atlantic and Pacific sources. America's largest single fish crop is the menhaden, 950,000,000 pounds of this material being processed in 1948. The Pacific sardine, or pilchard, is the third largest fish crop and is the second important source of condensed fish solubles. It was considered possible that menhaden and sardine solubles might differ in value as animal protein factor supplements, and the present investigation was undertaken to compare their relative potency by APF rat test.

EXTRACTION PROCEDURE

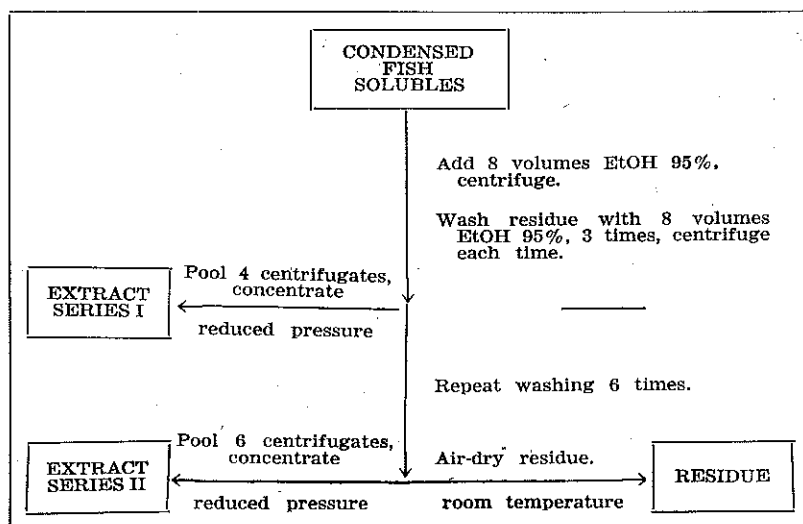


Fig. 3. Extraction procedure

A 150 g sample of condensed fish solubles from each of the two sources (a) was treated according to the scheme shown in Fig. 3. Raw materials and prepared fractions were tested with paired Sprague-Dawley albino rats during a 28-day post weaning growth period. All supplements were mixed directly in the diets. The isolated soybean protein used (b) was heat-treated (9) to nullify any possible nutritional effects attributable to antitrypsin (10). Dried distillery solubles (c) served to fortify the basal ration with

- (a)—The Borden Company.
 (b)—Archer-Daniels-Midland.
 (c)—Jos. E. Seagram and Sons.

respect to vitamin B₁₃ (11), known to be distinct from the animal protein factor in condensed fish solubles (3). Animals were housed individually in screen-bottom wire cages and were provided at 48-hour intervals with fresh food and water in excess. Animal protein factor activity was assessed as shown in Fig. 2, in terms of the percent increased gain of each test rat over its paired control. The results are summarized in Table II.

TABLE II*

APF ACTIVITY OF FISH SOLUBLES FRACTIONS

	APF ACTIVITY					
	MENHADEN			SARDINE		
FISH SOLUBLES	+	+	+	+	+	+
EXTRACT SERIES I	+	+	+	+	+	+
EXTRACT SERIES II		0			0	
RESIDUE	+	+	+			+

All supplements fed at 1% equivalence—dry basis.
 Six rat pairs in each test.

*Symbols defined in Fig. 2.

The potency of both menhaden and sardine solubles as sources of APF is evident from the data. It would appear, however, that the factor may occur in more than one form. The extracted residue from menhaden solubles carried considerably higher potency than did that from the sardine solubles, suggesting a greater amount of protein-bound activity in the former raw materials. It should be emphasized that interpretation of this point is restricted by the semi-quantitative nature of the test, and by the fact that supplements were fed at a single level. There is no doubt that a significant portion of the activity of each sample could be extracted with ethanol. Dry-matter determinations indicated approximately 10-fold concentration of activity in the extracts.

It has been reported by one group of investigators (12, 13) that the chick-growth factor in condensed fish solubles is insoluble in 95% ethanol, and other workers (3,4) that the rat-growth factor could be quantitatively extracted by the same solvent. In neither case was the species source of raw material disclosed. It would seem possible in the light of the present investigation that the former group may have been working with menhaden solubles containing a major proportion of bound activity, the latter group with sardine solubles containing most of its activity in extractable form.

A recent discussion of the animal protein factor activity of liver (14) has emphasized the possible multiple nature of this factor. In view of the clear-cut distinction made between zoopherin of liver (6) and the growth factor of alfalfa meal (15), and the recent announcement (16) identifying factor X (17), found in liver and alfalfa, with vitamin B₁₂, (18) the specific relation of vitamin B₁₂ to the animal protein factor remains obscure. Highly potent concentrates of the rat-growth factor in condensed fish solubles have been found to exhibit characteristics distinct from those of vitamin B₁₂ (3).

Further research is greatly needed to clarify the animal protein factor problem. The questions concerning purported APF activity of vitamin B₁₂, alfalfa meal and other dietary supplements remain to be answered through intensive studies using highly purified diets. The chemical and physical relationships between the biologically identical free and bound forms of the animal protein factor in condensed fish solubles likewise require elucidation.

Summary

Over 30% increased rate of gain is attained by weanling albino rats on a highly purified diet supplemented with condensed fish solubles. This animal protein factor activity is shown by condensed fish solubles prepared either from Atlantic menhaden or from Pacific sardines. A significant portion of the APF activity may be extracted from either source with 95% ethanol. Ethanol-insoluble activity is higher in menhaden solubles than in sardine solubles, suggesting presence of the animal protein factor in two forms, occurring in different ratios according to source.

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