A REVIEW OF THE CHEMICAL AND NUTRITIVE PROPERTIES OF CONDENSED FISH SOLUBLES

JOSEPH SOARES, JR.,[1] DAVID MILLER,[2] SUSAN CUPPETT,[2] AND PAUL BAUERSFELD, JR.[2]

ABSTRACT

This paper attempts to review some of the pertinent data available on the chemical and nutritive properties of condensed fish solubles with special reference to condensed menhaden solubles. Information concerning other kinds of fish solubles from various published sources is tabulated for comparison. The results of three analytical and three biological studies of menhaden solubles are reported.

Data show that menhaden stickwater contains an average of 4.8% protein, 93.4% water, and about 1.25% lipid. The solids are concentrated about seven times by vacuum evaporation to yield condensed solubles. Data show that this product contains about 32% protein, 49% water, and 11% fat. Additional data are presented on the content of amino acids in fish solubles, which show that the indispensable amino acid tryptophan is relatively low and, on the content of essential inorganic elements which are shown to be present, generally in acceptable levels. Fish solubles are shown to be rich sources of choline, niacin, pantothenic acid, riboflavin, biotin, and vitamin B_{12}.

An experiment with broiler chicks showed that the average metabolizable energy content of 10 samples of condensed menhaden solubles from commercial plants located along the Atlantic and Gulf coasts was 2.03 kcal/g on an "as fed" basis. Average protein and fat digestibility was 89 and 57%, respectively.

Further studies with broiler chicks showed that significant unidentified growth factor responses could be obtained when 5% menhaden solubles was used in diets. A significant response was obtained even when the chicks were reared in a gnotobiotic environment. This indicates that the presence of bacteria is not necessary for solubles to produce a growth response in chicks.

This review attempts to discuss some of the more pertinent recent published and unpublished data available on the chemical and nutritive properties of condensed fish solubles. For many years stickwater was considered as a waste effluent and was not used in animal feeds or in other manufacturing processes. However, with the postwar surge in production of fish meal, this by-product began to be recognized for its nutritional value. In the presynthetic nutrient era, condensed fish solubles was recognized as an outstanding source of B vitamins and minerals for poultry and swine. Later, solubles was the principle source of the "animal protein factor" or vitamin B_{12}. Today we still consider condensed fish solubles to be valuable for these purposes, but now more emphasis is put on its unidentified growth factor content.

There are various sources and kinds of condensed fish solubles available in the United States. However, about 80% of the solubles used in this country are produced by the menhaden reduction industry located along the Atlantic and Gulf coasts. Since 1963, the quantity of solubles available for animal feedstuff supplementation in the United States has averaged about 90,000 tons annually.

However, because of the difficulty in handling, storing, and mixing the viscous condensed fish solubles, a considerable quantity of this product is dehydrated on a carrier to produce a free-flowing dry product. One such product is "full

[1] College Park Fishery Products Technology Laboratory, National Marine Fisheries Service, NOAA; present address: Department of Poultry Science, University of Maryland, College Park, MD 20740.

[2] College Park Fishery Products Technology Laboratory, National Marine Fisheries Service, NOAA, College Park, MD 20740.

Manuscript accepted September 1972.

meal" containing 1 part condensed solubles to 3.5 parts of fish meal. This recombination of the two fish products is a logical approach as it is really equivalent to the original fish minus a major part of the original oil. Numerous other feedstuffs, such as soybean meal, alfalfa meal, or wheat middlings are also used to produce a free-flowing dry product consisting of one-half carrier and one-half dried condensed solubles.

PROCESS METHOD

Condensed fish solubles is one of two by-products produced by the wet reduction of whole fish to fish meal. Briefly, the production of condensed fish solubles by the "wet reduction" process involves cooking whole fish (e.g., menhaden) and subjecting the cooked fish to screw-type presses. The liquid fraction which passes through the screens of the presses is collected and separated by first passing through a vibrating screen to remove suspended material. The remaining liquor is either pumped into settling tanks for gravity separation or, more commonly, centrifuged to separate the oil and aqueous portion. Usually the centrifugation system consists of a three-phase centrifuge designed to separate oil, water, and suspended material. The aqueous portion in these centrifuges is collected in large storage tanks. During this storage process usually a quantity of sulfuric acid, or occasionally phosphoric acid, is added to the stickwater until a pH of 4.5 is reached (normal pH 6-7.0). The storage tanks are heated by steam coils to a temperature of approximately 150°F for 30 min to coagulate the proteins. The suspended and coagulated proteins are removed by centrifuges, and the free oil is removed by oil-separating centrifuges. The solid materials are dried on press cake and the liquid is called "stickwater." Table 1 shows the composition of several samples of stickwater produced in 1970. These samples contained an average of about 7% total solids and about 5% protein. The amino acid profile indicates that this protein is gelatinous in nature because of the relatively high glycine, proline, and alanine content.

Stickwater is then concentrated by means of vacuum evaporation, generally operated in units of three or more. Thus, the terms "triple effect" or "quadruple effect" are applied to the evaporation procedure. The evaporators may be horizontal or vertical, depending on the position of the steam tubes used for heat. Each evaporator consists of a series of tubes, usually vertical, holding the liquor and surrounded by steam. The tubes passing through the evaporator allow the vaporized water to escape at the top, and the partially evaporated product passes to the next unit at a lower position in the evaporator.

These evaporators are subjected to reduced pressure. The first unit is usually heated by low pressure exhaust steam from the boilers and is generally maintained at about 24 inches of mercury pressure (or under a slight vacuum). As the liquor reaches a predetermined concentration, it is drawn into the second unit. The vapors from the boiling liquor in the first unit are passed through the steam tubes of the second, and the liquor continues to boil by increasing the vacuum to about 28 inches of mercury.

In the third stage the process is either completed under a vacuum of about 30 inches of mercury or continued depending on the number of stages employed. When the liquor reaches approximately 50% solids, the process is finished and the solubles are stored. Thus, during the process the original material containing about 93% water is concentrated by a factor of at least seven. Other methods of condensing fish solubles have been and are used but not extensively enough to warrant further discussion here.

TABLE 1.—Chemical composition of Atlantic menhaden stickwater.[1]

Item	Amount	Item	Amount
	%		%
Protein (N × 6.25)	4.82	Valine	2.36
Moisture	93.43	Methionine	1.37
Ash	1.12	Isoleucine	1.59
Total fat	1.26	Leucine	3.34
Ether fat	0.83	Tyrosine	1.02
		Phenylalanine	2.12
	% nitrogen	Tryptophan	0.31
Ammonia	0.034	Taurine	3.55
		Asparctic acid	5.20
	% of protein	Serine	2.31
Lysine	4.45	Glutamic acid	8.22
Histidine	4.19	Proline	5.10
Arginine	1.89	Glycine	10.45
Threonine	2.06	Alanine	5.86

[1] Values are averages of data from three analyses.

TABLE 2.—Analytical data on condensed solubles.

Item	Menhaden solubles	Pacific sardine solubles	
	Lee (1956)	March (1962)	Lassen et al. (1951)
Dry solids, %	47.6 ± 3.5	50.3	50.0
Crude protein, % (N × 6.25)	33.5 ± 2.7	33.7	32.0
Ammonia, %	1.3 ± 1.1	--	--
Corrected protein,[1] %	27.2 ± 3.8	--	--
Fat, %	6.4 ± 3.2	4.3	7.0
Total ash, %	9.2 ± 2.0	9.2	12.0
Water-insoluble matter, %	4.5 ± 1.6	--	--
Water-insoluble ash, %	0.28 ± 0.21	--	--
pH	4.64 ± 0.54	4.5	--
Specific gravity	1.19 ± 0.03	1.2	--
Number of samples	32	?	?

[1] Corrected for ammonia-nitrogen 5.15.

COMPOSITION OF CONDENSED FISH SOLUBLES

Analytical data (Table 2) published by Lee (1956) showed that condensed fish solubles contained approximately 50% dry matter, which included protein (Kjeldahl nitrogen × 6.25), fat, minerals, and vitamins. According to the above author the major variable factors affecting composition were species and age of the fish, season and location of the catch, handling techniques, and type of plant equipment used during processing. Data describing other types of fish solubles produced in the United States are very scanty. However, Table 2 lists some average results for herring solubles reported by March (1962) and Lassen, Bacon, and Dunn (1951), and these appear very similar to the menhaden data.

Table 3 shows the proximate, total fat, and ammoniacal nitrogen composition of 24 condensed menhaden fish solubles samples collected during 10 months of the 1969 fishing season (Soares, Miller, and Ambrose, 1970). These samples were obtained from seven different fish meal plants located along the Middle Atlantic and Gulf coasts, and they represent regular commercial production. The methods for proximate analyses were all standard AOAC (Association of Official Analytical Chemists) methods except total fat, which is a chloroform-methanol extraction method of Bligh and Dyer that was modified by Smith, Ambrose, and Knobl (1964). Since the data from the Atlantic and Gulf samples did not differ significantly (although they are made from two different species of menhaden), it is presented together as an average. In general, these analyses indicate that the solids content is slightly more than 51%. The average concentration of protein (Kjeldahl N × 6.25) was 31.8% and that of ash was 7.8%. If these values are converted to a dry basis, they are very similar to those for menhaden fish meal reported by Kifer and Payne (1968) and Kifer, Payne, and Ambrose (1969). This is not to say that dried fish solubles is equivalent to fish meal in protein quality, but this only points out the relatively high protein content of this material even after allowing for nonprotein nitrogen (Table 3). The fat content, expressed on a dry basis is somewhat higher, however, than that found in fish meal. Ammoniacal nitrogen (which included volatile amines, etc.) makes up about 0.5% of the total nitrogen.

TABLE 3.—Proximate composition, total fat, and ammoniacal nitrogen content of menhaden fish solubles.[1]

Analyses	Average of 24 samples
	%
Protein (N × 6.25)	31.8 ± 2.52
Ash	7.8 ± 0.93
Ether fat	8.9 ± 2.13
Total fat	11.2 ± 2.09
Moisture	48.7 ± 2.18
Ammonia as % N	0.5 ± 0.23

[1] Soares, Miller, and Ambrose (1970).

PROTEIN

Biologically, the protein in solubles is considered inferior in quality because of its high content of gelatin, derived from fish collagen, which is low in the sulfur amino acids and almost completely devoid of tryptophan. Therefore, the essential amino acids content of the protein in condensed fish solubles is not adequate as a sole source of protein for the growth of chicks because tryptophan is deficient and the content of the other essential amino acids in condensed fish solubles is lower than that found in fish meal. Nevertheless, the protein may serve very well in a diet as a minor supplementary protein or in a diet otherwise adequate in amino acids since a 3% level of fish solubles contributes only 1% protein to the total diet.

AMINO ACIDS

Table 4 lists the amino acid composition of menhaden fish solubles published by Soares et al. (1970) and shows that solubles do contain some of the amino acids in reasonably high amount. All amino acids except available lysine, tryptophan, and cystine were analyzed by ion exchange column chromatography. Available lysine was determined by the method of Carpenter (1960). Tryptophan was determined by the method of Spies and Chambers (1948), and cystine was analyzed by a microbiological assay.

It is noteworthy that cystine averaged 1.05% of protein, which is slightly higher than the 0.9% of protein found in menhaden fish meal (Kifer and Payne, 1968). Glycine, an important amino acid in poultry rations since it is necessary for maximum growth of chicks, is the only other essential amino acid found in fish solubles at levels higher than in fish meal. As mentioned earlier, tryptophan is the first limiting amino acid based on chemical composition. Available lysine averaged about 83% of total lysine which is similar to the fish meal values that we have obtained. The taurine content is also shown, inasmuch as a recent report by Monson (1969) indicates that this amino acid may be one of the unknown growth factors. Table 4 also shows the amino acid composition of sardine and herring solubles. For the most part these data are similar to menhaden. However, some large differences exist in the histidine levels which are not explainable.

TABLE 4.—Amino acid content (% of protein) of various kinds of condensed fish solubles.

Amino acid	Menhaden solubles	Herring solubles[1]	Pacific herring solubles	Pacific sardine solubles
	Soares et al. (1970)	Laksesvela (1954)	Ewing (1963:291)	Lassen et al. (1951)
	% of protein			
Arginine	3.9 ± 0.65	5.2	4.5	4.3
Histidine	2.5 ± 0.80	0.7	6.0	5.8
Lysine	4.8 ± 0.56	4.3	4.3	4.9
Tyrosine	1.1 ± 0.20	0.7	--	--
Tryptophan	0.3 ± 0.07	0.1	0.4	0.4
Phenylalanine	2.1 ± 0.27	1.5	3.2	2.3
Methionine	1.6 ± 0.17	1.4	1.5	1.5
Cystine	1.1 ± 0.28	0.1	0.3	--
Threonine	2.1 ± 0.25	2.1	1.8	2.4
Leucine	3.7 ± 0.46	3.2	3.0	4.7
Isoleucine	1.9 ± 0.28	1.9	2.3	2.7
Valine	2.7 ± 0.35	2.5	3.4	3.0
Glutamic acid	8.1 ± 0.94	7.5	--	8.4
Glycine	8.7 ± 1.50	10.9	10.9	--
Proline	4.5 ± 0.60	--	--	6.7
Taurine	3.2 ± 0.50	?	--	--
Available lysine	4.0 ± 0.62	--	--	--
No. of samples	24	--	?	?

[1] Unknown origin.

MINERALS

The ash content of condensed solubles averages about 9% and occasionally is as high as 13% of total weight (Soares et al. 1970). The major inorganic elements (Table 5) are P, Na, and K.

TABLE 5.—Inorganic elements content of menhaden fish solubles.[1]

Inorganic elements	Average	Range	
		Low	High
	Percent	*Percent*	
K	1.57 ± 0.18	1.06	1.87
Na	1.14 ± 0.28	0.70	1.67
P	0.56 ± 0.14	0.20	0.80
Mg	0.11 ± 0.03	0.08	0.17
Ca	0.06 ± 0.02	0.03	0.11
	ppm	*ppm*	
Fe	573 ± 216.00	210.	1,000.0
Al	194.5 ± 224.00	16.0	640.0
Cu	44.3 ± 49.85	1.0	120.0
Zn	18.1 ± 7.93	4.0	31.5
Ba	5.25 ± 3.85	2.0	15.2
Mn	5.22 ± 3.37	2.0	14.2
Sr	3.73 ± 1.49	1.0	7.2
B	3.05 ± 1.64	1.0	6.4
Cr	2.86 ± 0.30	2.2	3.0
Se	2.4 ± 0.44	1.4	3.6

[1] Soares, Miller, and Ambrose (1970) and Soares and Miller (1970).

Chloride which is not included in Table 5 is also present in amounts about equal to Na. Others that are present in lower amounts include: Mg, Ca, Fe, Al, Zn, and Mn. The trace elements that are found at even lower levels include: Co, I, Ag, B, Ba, Cr, Li, Ni, Rb, Sr, Si, Se, and perhaps others not yet identified. Calcium and phosphorus analyses were determined by the method of Kingsley and Robnett (1958) as adapted for the Technicon. All other elements were determined by emission spectographic analysis.

These data show that the calcium and phosphorus levels are expectedly very low compared to fish meal since the major portion of these two elements are found in the bone which remains with the meal. Most of the other minerals in fish solubles also occur in lower concentrations than in fish meal. Selenium, however, tends to be found in concentrations that are equal to or greater than fish meal. Three minerals—aluminum, copper, and iron—were detected in quite variable concentrations. The average concentration of auminum in all solubles samples is somewhat lower than that reported by Kifer and Payne (1968) for fish meals from both the Atlantic and the Gulf coasts. The aluminum content of solubles from Atlantic coast plants was, however, considerably lower than that of menhaden fish meals, whereas the aluminum content of Gulf coast solubles was somewhat higher. Similar differences in aluminum content (unpublished data) were found in Gulf and Atlantic menhaden fish protein concentrate (FPC) made at the College Park Laboratory, starting with raw fish and using stainless steel equipment under aseptic conditions. Consequently, the higher aluminum content appears to occur in the Gulf species itself rather than as a contaminant after they are caught.

Selenium has become a focal point in trace mineral research since Schwarz and Foltz (1957) and Nesheim and Scott (1958) showed that it is essential for the prevention of dietary liver necrosis in rats and of encephalomalacia, exudative diathesis, and muscular dystrophy in chicks. Thompson and Scott (1968) have suggested that the range of 0.15 to 0.2 ppm is the desirable concentration for selenium in practical poultry diets. As little as 0.05 ppm selenium in the form of sodium selenite, however, has been found to prevent the physiological abnormalities mentioned above.

So far, the Food and Drug Administration has not permitted chemical compounds containing selenium to be added to the rations of livestock. For this reason, natural sources are currently the only available means of supplementing selenium in the diet. Fish meal has been shown to be a rich source of selenium by Kifer and Payne (1968) and Kifer et al. (1969).

A number of samples of fish solubles was obtained from commercial menhaden plants throughout the 1969 fishing season and analyzed for selenium. A total of 38 samples were analyzed—20 from fish meal plants along the Atlantic coast and 18 from plants along the Gulf coast.

Although neutron activation analysis was used to determine the selenium content of the fish meals reported in the above-mentioned papers, we found that this method did not give reliable results with fish solubles. Consequently, a wet chemical method (Hoffman, Westerby, and Hidiroglou, 1968) was used.

Table 5 shows the average results from all analyses. The data showed that there were higher levels of selenium in the Gulf product than in the Atlantic coast product (2.59 ppm vs. 2.22 ppm) (Soares and Miller, 1970). However, the corresponding fish meals from these two areas (Kifer et al., 1969) did not contain significantly different concentrations of selenium. This observation indicated to us that selenium is present in greater concentrations in the water-soluble portion of fish rather than in the water-insoluble solids, and the higher levels of selenium found in Gulf menhaden fish solubles may reflect leaching from the relatively high selenium soils draining into the Mississippi River or other local environmental conditions. Further research, however, may reveal other explanations for these differences.

Regardless of whether or not Gulf menhaden solubles or Atlantic menhaden solubles are used in feeds, a level of 2-2.5% will supply the minimal level of 0.05 ppm selenium in the diet based on total chemical analysis.

Availability determinations of selenium in fish meal and solubles are now in progress. To date there is some indication that this selenium source is not as available as sodium selenite (Miller and Soares, 1972; Scott, pers. Comm. to Morris and Levander, 1970).

VITAMINS

The fat-soluble vitamins are present in small quantities in condensed fish solubles. In contrast, the contents of the various water-soluble vitamins are at least 50% greater in the condensed solubles than in fish meal. Fish solubles are considered very rich sources for vitamin B_{12}, choline, niacin, and one or more unknown factors.

Table 6 lists data from six reports on the vitamin content of condensed fish solubles. The data are somewhat spotty and are often labeled "condensed fish solubles" with little or no details on its origin. The data are particularly deficient in regards to the fat soluble vitamins, inositol, pyridoxine, and folic acid. However, there seems to be reasonable reliability in the values given for the remaining vitamins. In particular, each kilogram of solubles contains about 4,400 mg of choline, 270 mg of niacin, 38 mg of pantothenic acid, 11 mg of riboflavin, 0.5 μg of biotin, and 440 μg of vitamin B_{12}.

NUTRITIVE VALUE

METABOLIZABLE ENERGY AND NUTRIENT DIGESTIBILITY

Metabolizable energy (ME) is the energy derived from a feedstuff after subtracting from the gross energy value the amount of energy lost

TABLE 6.—Vitamin content of condensed fish solubles.

Vitamin	Source[1]						Average
	1	2	3	4	5	6	
Choline, ppm	4,028.0	--	--	5,300.0	2,960.0	--	4,429.0
Niacin, ppm	169.0	307.0	325.0	230.0	308.0	--	268.0
Pantothenic acid, ppm	35.0	32.3	40.0	45.0	43.1	33.0	37.7
Folic acid, ppm	--	--	--	--	1.3	0.8	1.1
Inositol, ppm	--	--	--	--	352.0	--	352.0
Riboflavin, ppm	14.5	6.9	20.0	7.7	--	4.0	10.6
Pyriodoxine, ppm	--	8.3	--	--	--	34.0	21.2
Thiamine, ppm	5.5	2.9	4.0	--	--	5.0	4.4
Biotin, ppb	0.2	1.3	--	0.3	0.1	--	0.5
Vitamin B_{12}, ppb	--	640.0	--	400.0	550.0	180.0	443.0
Vitamin A, IU/g	--	360.5	--	--	--	5.8	183.0
Vitamin D, IU/g	--	55.0	--	--	--	--	55.0
Vitamin E, IU/g	--	--	--	6.0	--	--	6.0

[1] 1. National Research Council (1968).
2. Hideo, Murayama, and Yanase (1961).
3. Lassen, Bacon, and Dunn (1951).
4. Scott, Nesheim, and Young (1969:440).
5. Ewing (1963:291).
6. Murayama and Yanase (1960).

in the feces and urine. This parameter is universally accepted among poultry nutritionists as a valid estimate of dietary energy content and is very important in formulating least cost poultry rations.

Owing to the limited data available in the literature on the ME value of condensed fish solubles, Cuppett and Soares (in press) have conducted investigations concerning this property. Data were gathered on 10 of the menhaden solubles samples used in the analytical studies discussed earlier. The experimental design with a few modifications for convenience was similar to that used by Potter and Matterson (1960) for various common feed ingredients. In these experiments, eight 3-week-old White Rock broiler cockerels were placed in each battery pen. Each diet was fed to two groups of chicks for 5 days and excreta samples were collected for 3 days. Chromic oxide was incorporated in the diet at the 1% level as a marker. Fish solubles were fed at 0, 5, 10, and 15% levels. Analyses for proximate composition and gross energy were made on all diets and excreta samples.

The average ME value was 2.03 kcal/g which is somewhat higher than the calculated figures usually presented in feed analysis tables. These results are in good agreement with the data reported by Matterson et al. (1965) and Chu and Potter (1969) (Table 7). The ME value of all three reports averages 2.12 kcal/g (962 kcal/lb) with fat and protein digestibilities of 93.2 and 62.0%, respectively. It appears that much of the problem with lower calculated values reported in the various feedstuffs tables is due to underestimating the fat content of fish solubles.

TABLE 7.—Metabolizable energy and protein and fat digestibility evaluations of fish solubles in diets fed to young broilers and turkeys.

Source	Experimental animal	Metabolizamle energy	Fat digestibility	Nitrogen digestibility
		kcal/g	%	%
Matterson et al. (1965)	Chicken	2.094	--	--
Chu and Potter (1969)	Turkey	2.223	97.7	67.2
Cuppett and Soares (in press)	Chicken	2.030 ± 0.23	88.7 ± 2.48	56.2 ± 3.70
Average		2.12	93.2	62.0

UNIDENTIFIED GROWTH FACTOR(S)

The major contribution of condensed fish solubles is the unknown factor necessary for maximum growth of poultry and hatchability of eggs. Considerable evidence has been presented to demonstrate the existence of a stable unknown essential in condensed fish solubles. Combs, Arscott, and Jones (1954) indicated that the growth response obtained with arsanilic acid occurs only in the presence of the fish factors. The so-called fish factor is believed by Tamimine (1955) to be two components: one, organic in nature and the second, inorganic. Similar results were obtained by Steinke, Bird, and Strong (1963).

Combs et al. (1954) also reported a lack of chick growth response from fish solubles fed with penicillin. Similarly, Barnett and Bird (1956) found a lack of response with high levels of chloretetracycline. The conclusion is that the activity of the fish solubles occurs only in the presence of certain intestinal microorganisms. They also obtained no growth response when chicks were in new uncontaminated buildings. Actually, their bioassay method developed for evaluating the fish factor is dependent upon the ingestion of poultry excreta by the chicks on test. This would seem to confirm that beneficial bacteria flourish in the intestines of poultry only in the presence of the fish factor. Similar evidence exists for the essentiality of the fish factor for the hatchability of chicken and turkey eggs. However, there are some contradictory reports. The reason for the differences in the observations has not yet been elucidated.

TABLE 8.—Unidentified growth factor response from various sources of condensed fish solubles.

Reference	Animal	Fish solubles	Experimental period	Types of diet	Growth response
		%	*days of age*		*% of control*
Menge and Lillie (1960)	Chick	2	8-36	Conventional	105.0
Mason et al. (1961)	Chick	2	0-14	Conventional	122.0*
Mason et al. (1961)	Chick	2	0-28	Conventional	107.0*
Mason et al. (1961)	Chick	2	0-14	Purified	107.0
Mason et al. (1961)	Chick	4	0-28	Conventional	115.0*
Harrison and Coates (1964)	Chick	5	0-28	Conventional	113.0*
Harrison and Coates (1964)	Chick	5	0-28	All vegetables[1]	107.0*
Chu (1968)	Turkey	2-5	0-56	Conventional	106.0*
Harrison and Coates (1969)	Germ-free chick	5	0-28	Sterile conventional	104.0
Harrison and Coates (1969)	Conventional chick	5	0-28	Conventional	112.0*
Bhargava and Sunde (1969)	Chick	2	0-7	Purified	109.0*
Bhargava and Sunde (1969)	Chick	2	0-14	Purified	118.0*

[1] Only vegetable sources used.
* Statistically significant ($P<0.05$).

A compilation of recent reports (Table 8) in which the phenomena of unidentified growth factors in condensed solubles were studied brings out several interesting points. First, these data indicate that significant unidentified growth responses are still being observed even when every effort is made to supply all known nutrients in the experimental diets. Secondly, growth response seems to be maximal when the solubles content ranges from 2 to 5% of the diet and when these diets are fed to chicks for 14 to 21 days beginning with day-old birds. Growth responses tend to diminish as the birds become older. Furthermore, growth stimulations are consistently observed with battery-reared chicks fed conventional feeds or highly purified rations. Therefore, it is evident that stress, such as that found in field conditions, may not be necessary to obtain a significant growth response from feeding condensed fish solubles.

An interesting series of experiments conducted by Harrison and Coates (1964) showed that significant growth responses were obtained from chicks whose parents were maintained on conventional rations containing animal protein or all-vegetable rations. In fact, the average growth response was greater when the conventional diets were fed. In comparing the effects of feeding 5% solubles to conventional or germ-free chicks, a significant response was observed only with the conventional birds. This indicates that the presence of microbes does influence the magnitude of the growth response. However, these workers observed that the addition of a small amount of sterilized or fresh droppings to the diet caused a significant growth depression which could be counteracted by feeding fish solubles.

Miller and Soares (1972) have conducted some detailed experiments designed to study unidentified growth factors in fish solubles. They used virtually a complete chemically defined diet (Table 9) except for the sources of essential fatty acids, which were supplied as purified oils. The amino acid mixture was patterned after that of Dean and Scott (1965). The vitamins, minerals, and antioxidant were supplied in sucrose-based premixes (Miller and Soares 1972).

TABLE 9.—Basal composition for crystalline amino acid diet used to study unidentified growth factor responses in chicks.[1]

Ingredient	Amount
	g/kg
Sucrose	502.3
Crystalline amino acid[2]	225.3
Antioxidant premix[3]	2.0
Cellulose	80.0
Safflower oil	50.0
Menhaden oil	10.0
$CaCO_3$	5.0
$CaHPO_4$	26.9
Mineral mix[1]	28.5
Vitamin mix[1]	50.0
Choline Cl mix	20.0
	1,000.0

[1] Miller and Soares (1972).
[2] Dean and Scott (1965).
[3] Santoquin premixed one part with nine parts sucrose. Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

The diets in these experiments were balanced for energy, calcium, phosphorus, and total nitrogen. When fish solubles was added to the diets, the total nonessential amino acid content was balanced by adjusting the glutamic acid content, and the essential amino acid content was balanced by decreasing the amount of the respective crystalline amino acid in the diet. All additions of condensed fish solubles produced greater growth rates in chicks as compared to the controls.

One of the above experiments consisted of six treatments with four variables (Table 10) fed to day-old chicks (duplicate groups of 12) for 3 weeks. Diet 1 was our basic crystalline diet with no added protein whereas Diet 2 contained 5.0% fish solubles. A significant growth response was obtained by the addition of fish solubles. Diet 3 plus 0.5 ppm selenium (as sodium selenite) did not give any growth response over the control. Diet 4 containing 0.5 ppm selenium plus 0.82% proline (which Graber, Allen, and Scott (1970) suggest is an essential amino acid) gave a slight but nonsignificant response in growth. Similarly, Diet 5 with 0.5 ppm selenium, 0.82% proline, and 5% gelatin protein resulted in only a small growth response. Gelatin was incorporated in the diets because earlier experiments had shown that the presence of intact protein was beneficial to the overall growth response to crystalline amino acid diets. However, when gelatin and fish solubles (Diet 6) were incorporated in the diet, there again was a significant growth response that was similar to the response obtained with only fish solubles.

TABLE 10.—Growth response and efficiency of feed conversion of chicks fed purified diets supplemented with selenium, proline, gelatin, and fish solubles.[1]

Item	Diet					
	1	2	3	4	5	6
Se, ppm	0	0	0.5	0.5	0.5	0.5
Proline, %	0	0	0	0.82	0.82	0.82
Gelatin, %	0	0	0	0	5.0	5.0
Menhaden fish solubles, %	0	5.0	0	0	0	5.0
Weight gain[2]	[3]233	[4]279	[3]236	[3]247	[3]242	[4]286
Feed efficiency	0.62	0.58	0.64	0.64	0.67	0.54

[1] Miller and Soares (1972).
[2] Average weight (g) of 18 day-old chicks.
[3], [4] Means bearing different superscripts are statistically different ($P<0.01$).

In a further experiment by Miller and Soares (1972) Diets 5 and 6 as listed in Table 10 were irradiated with 4.5 Mrad of gamma radiation by the method described by Soares et al. (1971) and were fed to conventional and gnotobiotic White Rock chicks. The results (Table 11) of an initial experiment indicate that condensed fish solubles is capable of exerting a growth response whether or not bacteria are present. It should be noted that both groups of chicks were housed under conditions making it possible for the ingestion of excreta which most likely occurred because of the type feeders used. Therefore, these data may not be in conflict with those of Harrison and Coates (1969) who observed that germ-free chicks consuming sterile diets intentionally contaminated with sterile fecal matter exhibited growth responses when fish solubles was included in the diet whereas similar chicks fed diets with no fecal contamination showed no response to fish solubles.

SUMMARY

This review shows that condensed fish solubles (particularly those made from menhaden) is quite rich in many essential nutrients needed for livestock production. This is especially true if the composition is expressed on a dry-matter basis, since half of the product consists of water. Most essential minerals and water-soluble vitamins are found in solubles in relatively high concentrations when compared with other common feedstuffs. Fish solubles is highly digestible and rich in energy (measured as metabolizable energy) and, if expressed on a dry-matter basis

TABLE 11.—Growth response of chicks for purified diets containing selenium, proline, gelatin, and fish solubles.[1]

Environment	Menhaden solubles	Weight gain	Chicks
	%	*g*	*Number*
Diet 5			
Conventional	0	[2]69.6	16
Gnotobiotic	0	[2]69.5	6
Diet 6			
Conventional	5.0	[3]98.5	17
Gnotobiotic	5.0	[3]107.9	7

[1] Miller and Soares (1972).
[2], [3] Means bearing different superscripts are statistically different ($P<0.01$).

have a higher caloric density than fish meal. Currently the most important nutrient in condensed fish solubles is the unidentified growth factor because significant growth responses can be demonstrated with poultry and other species of livestock under field conditions as well as in the laboratory.

LITERATURE CITED

BARNETT, B. D., AND H. R. BIRD.
1956. Standardization of assay for unidentified growth factors. Poult. Sci. 35:705-710.

BHARGAVA, K. K., AND M. L. SUNDE.
1969. A short time chick assay for unidentified growth factors. Poult. Sci. 48:694-697.

CARPENTER, K. J.
1960. The estimation of available lysine in animal protein foods. Biochem. J. 77:604-610.

CHU, A. B.
1968. UGF(s), protein and fat digestibility, and metabolizable energy evaluations of fish solubles in diets of young turkeys. Ph.D. Thesis. Virginia Polytech. Inst., Blacksburg.

CHU, A. B., AND L. M. POTTER.
1969. Metabolizable energy, and protein and fat digestibility evaluations of fish solubles in diets of young turkeys. Poult. Sci. 48:1169-1174.

COMBS, G. F., G. H. ARSCOTT, AND H. L. JONES.
1954. UGF required by chicks and poults. 3. Chick studies involving practical-type rations. Poult. Sci. 33:71-79.

CUPPETT, S. L., AND J. H. SOARES.
In press. The metabolizable energy values and digestibilities of menhaden fish meal, fish solubles, and fish oil. Poult. Sci.

DEAN, W. F., AND H. M. SCOTT.
1965. The development of an amino acid reference diet for the early growth of chicks. Poult. Sci. 44:803-808.

EWING, W. R.
1963. Poultry nutrition. 5th ed. Ray Ewing Co. Pasadena, Calif., 1475 p.

GRABER, G., N. K. ALLEN, AND H. M. SCOTT.
1970. Proline essentiality and weight gain. Poult. Sci. 49:692-697.

HARRISON, G. F., AND M. E. COATES.
1964. Studies of the growth-promoting activity for chicks of fish solubles. Br. J. Nutr. 18:461-466.
1969. The effect of the gut flora on the growth response of the chick to fish solubles. Proc. Nutr. Soc. 28(2):70A.

HIDEO, H., S. MURAYAMA, AND M. YANASE.
1961. Fish solubles. I. Nutritive elements of commercial fish solubles. Takaiku Suisan Kenkyusho Kenkyu Hokoku 31:317-322.

HOFFMAN, I., R. J. WESTERBY, AND M. HIDIROGLOU.
1968. Precise fluorometric microdetermination of selenium in agricultural materials. J. Assoc. Anal. Chem. 51:1039-1042.

KIFER, R. R., AND W. L. PAYNE.
1968. Selenium content of fish meal. Feedstuffs 40(35):32.

KIFER, R. R., W. L. PAYNE, AND M. E. AMBROSE.
1969. Selenium content of fish meals II. Feedstuffs 41(51):24.

KINGSLEY, G. R., AND O. ROBNETT.
1958. Further studies on a new dye method for the direct photometric determination of calcium. Am. J. Clin. Pathol. 29:171-175.

LAKSESVELA, B.
1954. An unidentified chick growth factor in herring meal. Meld. S. S. F. 5:94-102.

LASSEN, S., E. K. BACON, AND H. J. DUNN.
1951. An evaluation of condensed whale solubles as a supplement in poultry nutrition. Poult. Sci. 30:422-425.

LEE, C. F.
1956. Preparation of a dry product from condensed menhaden solubles. U.S. Fish Wildl. Serv., Res. Rep. 45.

MARCH, B.
1962. Fish meal and condensed fish solubles in poultry and livestock feeding. *In* G. Borgstrom (editor), Fish as food. Vol. 2, p. 377-434. Academic Press, N.Y.

MASON, M. E., J. SACKS, AND E. L. STEPHENSON.
1961. Isolation and nature of an unidentified growth factor(s) in condensed fish solubles. J. Nutr. 75: 253-264.

MATTERSON, L. D., L. M. POTTER, M. W. STUTZ, AND E. P. SINGSEN.
1965. The metabolizable energy of feed ingredients for chickens. Agric. Exp. Res. Stn. Univ. Conn., Res. Rep. 7.

MENGE, H., AND R. J. LILLIE.
1960. Ineffectiveness of antibiotic combination on response of chicks fed fish solubles. Poult. Sci. 39:1188-1190.

MILLER, DAVID, AND J. H. SOARES, JR.
1972. A study of the methodology for assaying unidentified growth factor in fish meal and fish solubles. Poult. Sci. 51:1288-1291.

MILLER, D., J. H. SOARES, JR., P. BAUERSFELD, JR., AND S. L. CUPPETT.
1972. Comparative selenium retention by chicks fed sodium selenite, selenomethionine, fish meal and fish solubles. Poult. Sci. 51:1669-1673.

MONSON, W. J.
1969. Evidence that taurine may be one of the elusive unidentified factors. (Abstr.) Poult. Sci. 48:1846.

MORRIS, V. C., AND O. A. LEVANDER.
1970. Selenium content of foods. J. Nutr. 100:1383-1388.

MURAYAMA, S., AND M. YANASE.
1960. Nutritive elements of fish meal and solubles produced in various countries. Bull. Tokai Reg. Fish. Res. Lab. 27:55-59.

NATIONAL RESEARCH COUNCIL.
1968. Nutrient requirements of swine. Natl. Res. Counc. Publ. 1599. Natl. Acad. Sci., Wash., D.C.

NESHEIM, M. C., AND M. L. SCOTT.
1958. Studies on the nutritive effects of selenium for chicks. J. Nutr. 65:601-618.

POTTER, L. M., AND L. D. MATTERSON.
1960. The metabolizable energy of feed ingredients for chickens. Agric. Exp. Res. Stn. Univ. Conn., Prog. Rep. 39.

SCHWARZ, K., AND C. M. FOLTZ.
1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. J. Am. Chem. Soc. 79:3292.

SCOTT, M. L., M. C. NESHEIM, AND R. J. YOUNG.
1969. Nutrition of the chicken. M. L. Scott and Associates, Ithaca, N.Y.

SMITH, P., JR., M. E. AMBROSE, AND G. N. KNOBL, JR.
1964. Improved rapid method for determining total lipids in fish meal. Commer. Fish. Rev. 26(7):1-5.

SOARES, J. H., JR., AND D. MILLER.
1970. Selenium content of Atlantic, Gulf menhaden fish solubles. Feedstuffs 42(37):22.

SOARES, J. H., JR., D. MILLER, AND M. E AMBROSE.
1970. Chemical composition of Atlantic and Gulf menhaden fish solubles. Feedstuffs 42(33):65, 71.

SOARES, J. H., JR., D. MILLER, N. FITZ, AND M. SANDERS.
1971. Some factors affecting the biological availability of amino acids in fish protein. Poult. Sci. 50:1134-1143.

SPIES, J. R., AND D. C. CHAMBERS.
1948. Chemical determination of tryptophan. Anal. Chem. 20:30-39.

STEINKE, F. H., H. R. BIRD, AND F. M. STRONG.
1963. Unidentified chick growth factor in fish solubles. J. Nutr. 80:60-68.

TAMIMIE, H. S.
1955. The response of chicks to an unidentified growth factor in fish products. (Abstr.) Poult. Sci. 34:1224.

THOMPSON, J. N., AND M. L. SCOTT.
1968. Selenium in practical chicken feeds. Proceedings of the Cornell Nutrition Conference, p. 121-125.