Preparation of a Menhaden Hydrolysate for Possible Use in a Milk Replacer

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ABSTRACT—Milk replacers are protein sources that substitute for higher valued whole milk for calf feeding. A process, based on the mild hydrolysis of menhaden with pancreatin at pH 7.5, has been developed and yields a product with desirable milk replacer characteristics: high content and quality of protein, low mineral ash and residual fat, and complete solubility. The hydrolysate would be cost competitive with other milk replacer ingredients and represents a higher economic use for menhaden. The process could also be applied to presently underutilized species of finfish of the Gulf of Mexico and south Atlantic areas.

INTRODUCTION

There is a large commercial market for milk replacer formulations in the United States and abroad. Milk replacers are complete rations that are substituted for whole milk in the feeding of calves and other newborn animals (e.g., lambs and pigs). They are of particular value in dairy herds for the feeding of replacement animals, veal calves, and calves which are fed for beef production.

Dried skimmed milk, once the major source of protein in many milk replacer formulations, has been largely replaced by dried whey or casein because of the cost advantage. In recent years, soy proteins have found increased use as an ingredient in combination with milk protein.

The criteria for a top quality milk replacer ingredient (Bauersfeld and Soares, 1972) are: 1) Cost advantage over dry skim milk; 2) consistent quality and chemical composition (80-90 percent protein, less than 1 percent fat, less than 10 percent ash, less than 8 percent moisture); 3) high protein availability and biological value; 4) good suspendability in liquid diet; 5) no strong odor; light in color; 6) low bacterial count; 7) storage stability; and 8) dependable, year-round supply.

There have been several studies on the use of fish protein products as milk replacer ingredients. Huber (1975) reported that fish protein concentrate (FPC) could replace 35 percent of the milk proteins in formulations fed to calves less than 3 weeks old and up to 70 percent of the total protein for older calves, with good results. Huber and Slade (1967) reported successful results when milk replacer diets contained up to 40 percent of the total protein from defatted fish meal, although growth was depressed at levels of 60 percent and above. Bauersfeld and Soares (1972) obtained good results with a replacer diet containing 10 percent condensed fish solubles (50 percent dry matter), but a diet containing 15 percent freeze-dried solubles (equivalent to 30 percent condensed solubles) was found to depress the growth rates of young lambs.

Fish protein is not commercially used in milk replacers in the United States, but significant amounts have been used in milk replacers in Europe. Astra Nutrition[1] of Sweden has sold an FPC product called "Prot-Animal" for use in milk replacers. Although it has protein of high nutritive quality, it has poor suspendability in liquid diets. Two companies of France, however, have developed processes for the production of fish protein hydrolysates with good suspendability for use in milk replacers. Nacoma (of Nantes) has trawlers equipped with shipboard processing equipment for the enzymatic hydrolysis of filleting waste and trash fish. Sopropeche of Boulogne-sur-Mer has been more successful and, according to Tatterson and Wignall (1976), expanded the capacity of their production facilities from 2,000 tons/year to 8,000 tons/year of milk replacer product in 1973. The European market for such fish protein products has been depressed recently by an oversupply of dried skimmed milk, at artificially low prices, due to government subsidies.

The milk replacer market offers an interesting potential for the upgrading of menhaden products to a higher valued product. As fish meal, menhaden sells on a protein equivalent basis for approximently 40¢/pound, while dried skimmed milk is worth about $1.20/pound of protein. The relative costs of several milk replacer ingredients, including cost estimates for the enzymatic hydrolysate of menhaden prepared in this study, are listed in Table 1. Cost estimates were made using a computer program that estimates detailed equipment, "total capital," and operating costs for several different FPC processes (Almenas et al., 1972). It was estimated that the menhaden hydrolysate could be produced for 30¢ to 36¢/pound and sold for 50¢ to 65¢/pound to

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[1]Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1.—Milk replacer ingredients—relative costs.

Ingredients	Cost (¢/lb)	Percent protein	Protein cost (¢/lb)
Dried skim milk	41	35	117
Dried whey	8	12	67
Casein	58	90	64
Sodium caseinate	75	93	81
Soy protein	13	50	26
Menhaden hydrolysate			
Low estimate	50	83	60
High estimate	65	83	78

yield an annual after-tax return on total capital investment of 10 percent. Both high and low estimates were based on an assumption of a plant processing 200 tons/day of fish at 3¢/pound, using fuel at 10¢/therm (or 100,000 BTU), electricity at 3¢/kwh, and labor at $5/man-hour. The high estimate was based on 150 days of operation per year and the low estimate on operations for 250 days/year.

PROCESSING STUDIES

In earlier work at the College Park Laboratory of the NMFS Southeast Fisheries Center, several forms of fish protein were evaluated for possible use in milk replacers. Hexane extracted fish meal and FPC were found to have excellent nutritive value but very poor suspendability. Acid and alkaline hydrolysates and a lactobacillus ensilage were relatively poor nutritionally. An enzymatic hydrolysate of menhaden press cake was most promising for use in milk replacers based on a combination of good suspendability and an acceptable nutritive value. Therefore, it was decided to focus on the development of an enzymatic hydrolysate of menhaden or menhaden press cake for possible use in milk replacer formulations.

Initially, both Type A and Type B hydrolysates were investigated. In the Type A process all insoluble solids remaining after hydrolysis are removed by screening and centrifugation. A totally soluble, low-fat powder is recovered by spray drying. Type B is a whole slurry product, liquified by enzymes and screened for removal of bones and scales. It can be produced fairly cheaply, and in high yields, but a high fat content, dark color, and strong odor make it unsuitable for use in milk replacers. To meet the requirements for a first-class milk replacer, it was decided to further evaluate the Type A process.

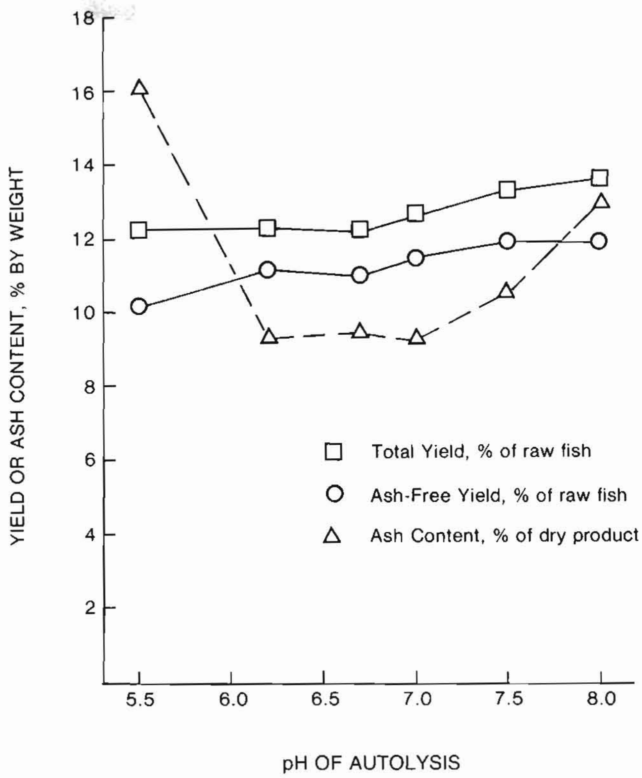

Figure 1.—Yield and ash content versus pH of autolysis of menhaden.

Use of an alkaline bacterial enzyme at pH 8.5 was recommended by Hale (1974) for the preparation of a soluble FPC with good yield, amino acid profile, and nutritive value. However, the soluble ash content of the product was higher than desired for milk replacer use because of the required pH adjustment.

Figure 1 shows the effect of pH of autolysis on the ash content of the dry product as well as total and ash-free yields of the soluble product. Yields are expressed on the basis of the initial weight of raw fish used. Menhaden was autolyzed at six different pH levels ranging from 5.5 to 8.0. Results indicated that at pH 7.5 the ash-free yield was highest and the residual ash content acceptable.

Autolytic activity varies with different catches of fish and a commercial proteolytic enzyme preparation should be added to assure a good reaction rate and yield of soluble product. Pancreatin was more effective than other enzymes tested for hydrolysis of menhaden at pH 7.5. It was also discovered that menhaden hydrolyzed with pancreatin after an initial pH adjustment to 7.5 with calcium hydroxide could be clarified by centrifugation, after hydrolysis, without acidification. This resulted in a soluble product with an ash content after drying of less than 10 percent.

THE PROCESS

The hydrolysis process is outlined in Figure 2. Raw menhaden was ground through a Hobart meat chopper and mixed with an equal weight of preheated water. Calcium hydroxide was added to raise the initial pH from about 6.6 to 7.5. The proteolytic enzyme, pancreatin (4×NF), was added at a level of 0.06 percent of the wet weight of fish. The slurry was agitated con-

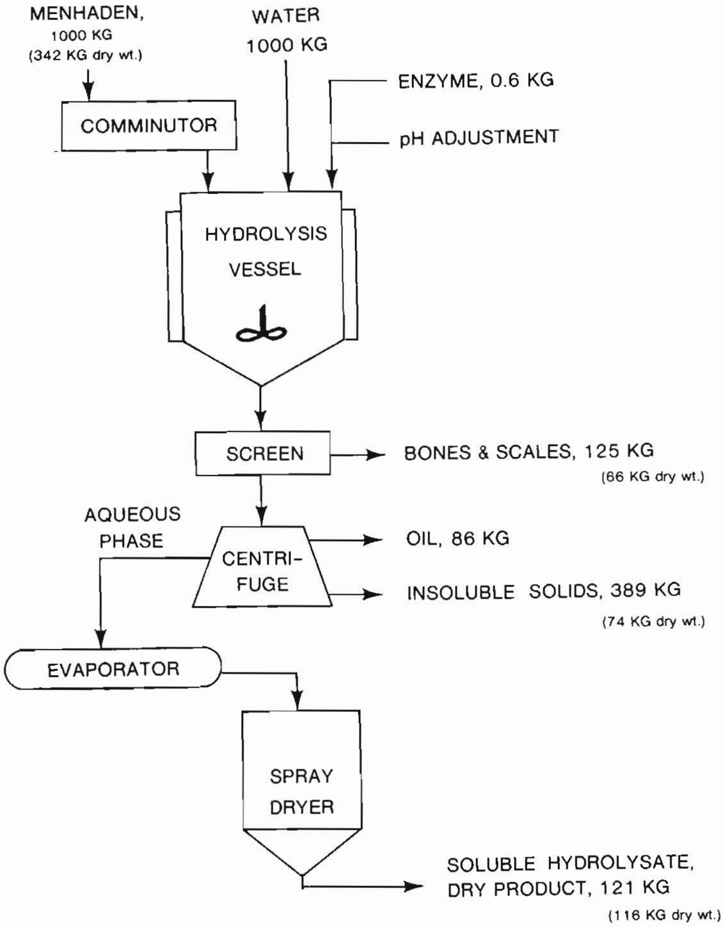

Figure 2.—Hydrolysis process for milk replacer ingredient from menhaden.

tinuously at 52°C (126°F). After 1 hour the pH fell to about 7.0 and was readjusted to 7.5 with NaOH. After a total hydrolysis period of 3 hours at 52°-53°C, the hydrolysate was passed through a 40-mesh screen to remove bones and scales. Insoluble solids and fat were then removed by centrifugation. A final clarification by filtration is optional. The clarified hydrolysate was spray-dried to yield a cream colored and completely soluble product.

Twelve runs were made using the 6-liter glass jars of a New Brunswick fermentor system. A series of additional runs were made with 60-pound batches of menhaden in a jacketed kettle with an air-driven agitator. After removal of insoluble sludge with a Fletcher solid bowl centrifuge, the hydrolysate was heated to 80°C (176°F) and passed through a small DeLaval cream separator to remove free oil. The dry product was then recovered using a Bowen laboratory model spray dryer. The average yield of dry powder was 11 percent of the wet weight of fish.

Although most runs were of necessity made with frozen menhaden, two runs were made with fresh, iced menhaden, and normal results were obtained with no problems in regard to sedimentation or yield. In one pair of runs, chlortetracycline was added to one batch at 250 ppm, but no difference in total bacterial plate counts (TPC) was observed. Hydrolyzed slurries had TPC's of about 500/g and the concentrated, spray-dried products had about 10,000/g.

THE PRODUCT

The spray-dried product satisfies most of the requirements for a first-class milk-replacer ingredient. It is a totally soluble cream powder with about 83 percent protein, less than 10 percent ash, and less than 1 percent total fat. Being moderately hygroscopic the product requires moisture-proof packaging. Proximate analyses are listed in Table 2 for the raw menhaden, enzymatic hydrolysate, and by-product streams of bones and insoluble sludge. The average amino acid analysis for two composite samples, representing a total of eight hydrolysate batches, is shown in Table 3. Calculation of chemical scores (Rama Rao et al., 1959) indicates that the sulfur amino acids (methionine and cystine) are first limiting and isoleucine second limiting nutritionally.

Table 2.—Proximate analyses (percent) for menhaden, hydrolysate, and by-products.

Item	Moisture	Protein	Fat	Ash
Raw menhaden	65.77	14.85	14.91	4.25
(Dry basis)		(43.66)	(43.84)	(12.50)
Bones and scales	47.12	16.93	4.47	27.79
(Dry basis)		(34.42)	(9.09)	(56.50)
Insoluble solids	81.14	9.63	7.65	3.52
(Dry basis)		(46.30)	(36.78)	(16.92)
Soluble product	6.51	82.77	0.80	8.67
(Dry basis)		(89.73)	(0.87)	(9.40)

Table 3.—Amino acid analysis for menhaden hydrolysates.

Amino acid	Average percent of sample weight	Average percent of protein (g/16 g N)
Lysine	7.05	8.45
Histidine	1.79	2.14
Ammonia	1.06	1.28
Arginine	4.95	5.94
Taurine	0.98	1.18
Aspartic acid	10.78	12.92
Threonine	3.51	4.21
Serine	3.34	4.01
Glutamic acid	11.66	13.97
Proline	3.49	4.19
Glycine	5.68	6.81
Alanine	5.51	6.61
Valine	4.20	5.04
Methonine	2.07	2.48
Isoleucine	3.46	4.15
Leucine	6.23	7.47
Tyrosine	2.53	3.03
Phenylalanine	2.93	3.52
Tryptophan	0.95	1.14
Cystine	0.79	0.96
Total	82.96	99.50

The nutritive value of our laboratory-produced pancreatic hydrolysate of menhaden has been determined in several rat feeding trials. Male weanling rats were placed on diets containing 10 percent protein contributed by the test ingredient and diets were formulated to contain sufficient amounts of all other essential nutrients. Rats were fed ad libitum during a 4-week test period. The results of feeding trials with a composite of spray-dried products from four hydrolysate batches (PCR 14-17) are shown in Table 4. The protein efficiency ratio (PER) of the menhaden hydrolysate was at least equal to the casein control diet. All data were analyzed statistically, utilizing a one-way analysis of variance and the Student-Newman-Keuls multiple-range test with $P = 0.05$ set as the level of significance (Steel and Torrie, 1960).

Table 4.—Results of 4-week rat feeding trials evaluating menhaden hydrolysate, PCR 14-17.

Item	*n*	Weight gain ± SE (g)	[1]PER ± SE
		Experiment no. 95	
Casein control	30	124 ± 2.5	3.26 ± 0.032
Menhaden hydrolysate	9	130 ± 4.3	3.24 ± 0.069
		[2]*Experiment no. 99*	
Casein control	9	107^b ± 2.3	3.02^b ± 0.066
Menhaden hydrolysate	10	120^a ± 3.8	3.30^a ± 0.060

[1]PER = protein efficiency ratio.
[2]Means within a column with different letter superscripts (a, b) are significantly different ($P < 0.05$).

DISCUSSION

The hydrolysis process we have described could best be carried out in conjunction with fish meal processing. It is a relatively mild hydrolysis, and the considerable residue of insoluble solids could be returned to the fish meal process. For an independent processing plant, the insoluble solids and bones would be drum dried and milled to produce an animal feed product.

The pancreatin enzyme was chosen because of its effectiveness at pH 7-7.5 with a resulting low soluble-ash content in the product. An alkaline bacterial protease would be more cost effective at present prices if used at pH 8-8.5, but it would increase the product's ash content. If acidification should be required for proper centrifugation in industrial processing, this would also result in a higher soluble ash content in the final hydrolysate product.

On a protein equivalent basis, the projected cost for the menhaden hydrolysate (Table 1) is only about half as much as dried skimmed milk and is quite competitive with dried whey and imported casein. Soy protein is cheaper, but requires additives for proper suspendibility and the maximum amount that can be used may be limited by nutritional factors, such as amino acid profile or excessive starch content. An effective commercial formulation would probably include both menhaden hydrolysate and soy protein.

The menhaden hydrolysate has a fairly high PER value and supports good growth in small animal feeding trials, but a large-scale calf feeding trial is necessary to establish its value for use in milk replacer formulations. Therefore, the National Marine Fisheries Service is now funding such a study. The menhaden hydrolysate being tested was prepared through the cooperation of the National Fish Meal and Oil Association, with the Zapata-Haynie Corporation, Reedville, Va., supplying plant processing equipment and manpower.

Although fish meal prices have risen to a profitable level, future increases in production costs could possibly make fish meal too expensive for continued use at presently recommended levels. The milk replacer market has a potential for absorbing suitable products derived from menhaden (or other industrial fish) at a higher economic value.

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