in myosin in the interaction of myosin with actin, and suggests that the increase in Ca^{2+}-ATPase activity is a universal feature of mammalian skeletal muscle which has undergone some changes during the storage under denaturing conditions. To detect whether or not a local conformational change of myosin in the actin-myosin complex system takes place, measurement of EDTA-ATPase at high ionic strength may be recommended as the best method.

This study provides us with a new approach through which the mechanism of modification of actin-myosin interaction during postmortem storage of skeletal muscle, which has been suggested independently by many workers (Fujimaki et al., 1965a; Gothard et al., 1966; Takahashi et al., 1967; Stromer et al., 1967), will be clarified. Along these lines, investigations on Ca^{2+}- and EDTA-ATPase activities of the myosin-actin/troponin-tropomyosin complex system are now under way in our laboratory.

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Received for review April 14, 1975. Accepted July 16, 1975.

Nutritional Evaluation of Protein from Shrimp Cannery Effluent (Shrimp Waste Protein)

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The protein efficiency ratio, PER, was determined for three proteins, casein, ISP (isolated soybean protein), shrimp waste protein, SWP, collected and processed from shrimp cannery effluent, and a mixture of equal proportions of SWP and ISP. With albino rats as the test animals, four isocaloric, isonitrogenous diets (4045 cal/g; 1.6% nitrogen) containing one each of the above protein sources were used to evaluate the effects of SWP on five criteria: food intake, body weight, PER, liver weight, and the ratio of protein to fat in the liver. SWP promoted rat growth 80% as efficiently as casein; moreover, SWP improved protein quality 74% in a soybean diet when SWP replaced half the soybean protein in the diet. A projection of potential practical applications would include incorporation of SWP in canned or processed pet foods and/or use of SWP as an animal feed supplement in poultry or livestock rations, in addition to reduction of water pollution.

Holder (1950) and Novak (1970) stated that protein from fishery products and by-products such as fish meals, condensed fish solubles, fish protein concentrate, and proteins from crude waste meal of crab and shrimp are excellent sources of protein because of their higher values in protein content and quality. Sure and Easterling (1952) found that the amino acid constituents of fish protein are of particular importance when incorporated into a diet of plant protein such as zein, soybean protein, cottonseed meal, and gluten. Also, Combs (1961) and Winchester (1963) indicated that fish proteins can substitute the deficiency in amino acids of plant proteins, which fail to provide the minimal requirements of the essential amino acids for growth and biologi-

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Table I. Proximate Composition of Protein Sources

Protein	% Moisture	Ash	Protein	Ether extract	Crude fiber	Calcd value, cal/g
ANRC casein[a]	3.81	2.34	91.48	0.01	0.00	5486
ISP[b]	4.50	3.52	92.31	0.00	0.01	5347
SWP[c]	10.00	6.33	58.98	16.97	1.62	5170

[a] ANRC casein from Sheffield Chemical Co., Norwich, N.J. Protein factor is 6.38. [b] Soybean protein isolate (Promine-D) from Central Saya. Protein factor is 6.25. [c] Shrimp waste protein. Protein factor is 6.25. All previous analyses are the mean of three aliquots except the SWP mean is for 12 aliquots. Total bacterial count for SWP is less than 200 organisms/g.

Table II. Amino Acid Profile of Protein Sources

Amino acid	g/16 g of N Casein	SWP	ISP[a]
Essential			
Half-Cys	0.76	1.59	1.2
Ile	6.55	3.26	4.9
Leu	10.05	7.57	7.7
Lys	8.01	6.17	6.1
Met	3.08	2.84	1.1
Phe	5.39	4.56	5.4
Thr	4.28	4.28	3.7
Trp	1.33	1.26	1.4
Val	7.39	4.42	4.8
Nonessential			
Ala	3.35	5.29	3.9
Arg	4.07	6.31	7.8
Asp	7.39	10.74	11.9
Glu	23.05	15.46	20.5
Gly	1.99	4.29	4.0
His	3.02	1.90	2.5
Pro	11.75	3.44	5.3
Ser	6.65	4.53	5.5
Tyr	5.82	3.64	3.7

[a] Available from data furnished by Central Soya (Promine-D).

cal functions for animals from dairy cows to trout. Holder (1951) in other biological studies stated that both growth and biological functions depend on the quality of the protein as amino acid constituents rather than absolute percentage of protein.

Numerous articles have discussed the nutrition evaluation of protein concentrate and other fishery products and by-products. Only those studies pertinent to this study have been selected.

Kwee et al. (1969) supplemented pasta (rice, corn, soybean, tapioca, and other vegetables) with FPC at 10–20% levels. All diets showed a significant increase in PER values when supplemented with FPC. Stillings et al. (1971) tested the nutritive value of wheat flour fortified with either FPC or lysine in diets at different levels of supplementation (FPC from 0.0 to 25% and lysine from 0.1 to 1.0%). Fifteen percent FPC when added to the diet (1.6% N) as a source of protein and supplemented with 0.2 to 0.4% lysine gave the maximal PER values, in contrast to those diets (1.6% N) supplemented with lysine only.

A similar study by Parkins et al. (1968) showed that mixing wheat flour with fish flakes at the ratios of 7:1 to 12:1 and feeding to test animals (rats) provided a 22 to 27% protein level depending on the type of wheat used. PER values were reported as 2.14–2.51 for the various diets, 2.50 for casein, 3.30 for fish flakes diet, and 0.39 for the gluten.

Dubrow and Stillings (1971), Stillings (1967), and Stillings et al. (1969) suggested that FPC should be supplemented with cystine and methionine, which are considered as the two limiting amino acids, especially if prepared with the use of high temperature where destruction of the two amino acids occurs. Maximal PER values were obtained in diets supplemented with FPC when methionine was added in adequate amounts (0.2%) to meet the requirements for growth and biological functions as recommended by the National Research Council (1962) and Rama Rao et al. (1964). Wilgus (1958) suggested that better biological efficiency of diets for nonrumen animals of all ages would be achieved if diets are formulated on the basis of amino acid adequacy rather than protein level. Draper and Rhian (1942) found that the excess of amino acids in diets might depress growth or cause other amino acids to be required for maintaining a balanced ratio in the formulated diet, if maximal growth and proper biological functions are to be achieved. Sidwell et al. (1970) found that addition of fish protein concentrate to diets containing soybeans increased the value of soybean diets for growth and biological functions (PER values for FPC mixed with soybean ranged from 2.96 to 3.25 for various species of fish as compared with the value of 3.00 for the casein diet).

EXPERIMENTAL SECTION

Sample Collection and Analysis. From the discharge pipe of a shrimp cannery (Robinson Canning Co., 1970), a representative volume of 2650 l. of effluent was collected throughout the processing period. The processed shrimp (*Penaeus aztecus*) were from the Gulf of Mexico. The entire volume was brought to the isoelectric point (pH 4.4–4.7) by the addition of approximately 175 ml of concentrated technical grade hydrochloric acid (which contained 30% hydrogen chloride) to settle the suspended proteinaceous material. The proteinaceous material was separated and dried by means of a small counter-current double drum drier (type 053-VIM-18, Reliance Electric and Engineering Co.) with direct steam at 33–35 psi (256–260°F). The final dried material of shrimp waste protein (SWP) was subjected to proximate analyses according to AOAC methods (1970) and its total bacterial count was less than 200 organisms/g (Difco Laboratories Inc., 1969) as shown in Table I. Amino acid profiles for SWP and casein were conducted in an earlier study (Toma and Meyers, 1975) as shown in Table II.

Nutrition Evaluation. Shrimp waste protein (SWP) was used in this study in comparison with other protein sources usually used for experimental animals. These proteins are ANRC casein and isolated soybean protein (ISP) [(Promine D) Central Saya, Chicago, Ill.]. The proteins were used in the formulation of four diets according to AOAC methods (1970) as one batch preparation for each diet, as shown in Table III. The diets were: diet 1, ANRC casein; diet 2, SWP; diet 3, ISP; diet 4, a mixture consisting of equal parts by weight of SWP and ISP (for every 16 g of nitrogen, SWP provides 6.24 g and ISP provides 9.76 g).

Table III. Interrelationship and Computed Proportions of Ingredients in the Four Diets

Nitrogen source	Interrelationship, g/100 g diet	g of ingredient/kg of diet: 1 Casein	2 SWP	3 ISP	4 SWP–ISP mixture
Protein sample	X to provide 10 g of protein	115.76	169.49	108.33	132.23
Cottonseed oil	$8 - ([X \times \%$ ether extract[a]$]/100$	79.99	51.24	80.00	68.79
Salt mixture	$5 - ([X \times \%$ ash$]/100)$	47.29	39.27	45.10	43.49
Vitamin mixture[c]	1	10.00	10.00	10.00	10.00
Cellulose	$1 - ([X \times \%$ crude fiber$]/100)$	10.00	7.25	10.00	8.92
Water[b]	$5 - ([X \times$ moisture$]/100)$	45.59	33.05	45.13	40.41
Corn starch	To make 100	691.37	689.70	701.44	696.16

[a] All percentage figures refer to the proximate composition of the sample. [b] On the basis of proximate analysis the diets were equalized with respect to moisture, fat, ash, and crude fiber. [c] All diets contained vitamins and minerals as specified in AOAC (1970).

Table IV. Nutritional Evaluation of Rats Fed Four Test Diets

Diet no.	Protein source	Weight gain, g	Food consumption, g	PER, g of wt gain/g of protein consumed
1	Casein	132.1 ± 11.3[b]	421.9 ± 21.3	3.13 ± 0.15
2	SWP	91.7 ± 14.2	369.1 ± 36.8	2.48 ± 0.21
3	ISP[a]	25.6 ± 11.2	255.3 ± 36.4	0.96 ± 0.38
4	SWP–ISP	51.8 ± 7.5	307.8 ± 31.9	1.68 ± 0.12

[a] Of the ten rats that received the ISP diet, nine of them gained weight. One rat, initial weight 70 g, lost 8 g during the first 3 days of the assay period, and thereafter its body weight fluctuated between 61.5 and 70 g. This atypical animal consumed 183.5 g of the ISP diet during the 28-day assay period. If the data for this rat are omitted, the mean values for weight gain, food consumption, and PER, calculated from the ISP data of the other nine rats, are, respectively: 28.4 ± 7.0; 263.3 ± 27.9; and 1.07 ± 0.19. [b] Standard deviation of the mean.

Each diet was isocaloric and isonitrogenous (4045 cal/g; 1.6% nitrogen), based on Parr Bomb calorimeter and Kjeldahl determinations, respectively.

The protein efficiency ration procedure was carried out under the standardized conditions of protein level, species of test animal, age of animal, use of appropriate duration of assay, and method of feeding as specified in AOAC (1970).

Test Animals. Four test groups of ten weanling, male albino rats of the Carworth Farms CFE strain were used. There was a 3-day acclimation period (fed on a casein diet only at 8.5 g/rat per day) for all rats. The animals were housed individually in screen bottom cages in an air conditioned room, thermostatically controlled at 23°. Assigned diets and distilled water were offered daily ad libitum. Food intake and weight gains of each animal were recorded every 3 days for 28 days. Each group received one of the four test diets. Groups 1, 2, 3, and 4 of the rats received, respectively, diets 1 (casein), 2 (SWP), 3 (ISP), and 4 (SWP–ISP mixture).

At the end of the assay period the rats were sacrificed with chloroform. Their livers were removed and weighed, and the ratio of liver weight/body weight calculated and recorded for each rat and for each group.

The rat livers were combined into four groups, each of which contained ten livers according to the diets the animals had received during the assay period and freeze-dried. The ten livers in each group were pulverized with a glass rod in a 27 × 100 mm o.d. Pyrex dish, for determination of moisture, fat, and protein according to AOAC methods (1970).

RESULTS AND DISCUSSION

From the amino acid profile listed in Table II, it appeared that SWP was superior to casein in cystine and arginine, equal in threonine, but less than casein in regard to the rest of the essential amino acids. Initially these findings would suggest that SWP would not promote growth in rats

Table V. Analysis of Variance for Protein Efficiency Ratios

Source of variation	df	Mean square	F value: Calcd	F value: df
Total	359			
A. Diets	3	64.1310	162.89[a]	3/36
B. Time (periods)	8	6.0125	15.45[a]	8/288
AB. Diets × time	24	1.2195	3.13[a]	24/288
In error term A (for diets)			Sum of squares	
R. Rats	9		3.0536	
RA. Diets × rats	27		11.1200	
Error			14.1736	
14.1736/36 = 0.3937 = mean square for error A (diets)				
In error term B (for time and diets × time)				
RB. Rats × time	72		33.7882	
RAB. Rt × Dt × Tm	216		78.2629	
Error	288		112.0511	
112.0511/288 = 0.3891 = mean square for error B (time and diets × time)				

[a] $P < 0.01$.

Table VI. Data of Rat Liver

Dietary	Liver wt, g	Ratio liver wt/ body wt, %	Protein, % of liver D.M.	Fat, % of liver D.M.	Ratio, protein/fat
Casein	10.8[a] ± 1.2[b]	5.25 ± 0.51	66.50[c]	6.3[c]	10.4
SWP	10.0 ± 1.7	6.07 ± 0.62	59.05	8.01	7.4
ISP	4.8 ± 0.4	4.93 ± 0.48	60.92	10.71	5.7
SWP–ISP	6.7 ± 0.8	5.42 ± 0.55	60.91	11.28	5.4

[a] Mean of ten livers' weights, liver weight of a control at beginning of the experiment was 3.1 g. [b] Standard deviation of the mean. [c] Mean of three aliquots. D.M. = freeze-dried matter.

as efficiently as casein. Because of the increasing importance of soybeans in the agricultural economy of Louisiana, isolated soybean protein (ISP) was selected for comparison in this study. From Table II, with the exception of tryptophan and phenylalanine, it can be seen that ISP contained less of the other essential amino acids than did casein. These differences reflect that ISP, like SWP, would not promote growth in rats as efficiently as casein; consequently, the SWP–ISP mixed diet will follow suit.

To test the nutritional value of SWP per se and/or in combination with plant proteins as compared with those done on FPC [Kwee et al., 1969; Parkins et al., 1968; Dubrow and Stillings, 1971; Stillings, 1967; and Stillings et al., 1969] the protein efficiency ratio (PER) was conducted and the results are shown in Table IV. These values indicate that SWP, ISP, and the SWP–ISP mixture were efficient in promoting growth in rats by 80, 31, and 54%, respectively, as compared to casein. These differences indicate that ISP, like SWP, would not promote growth in the rat as efficiently as casein. The PER values of SWP were similar to the findings by Sidwell et al. (1970).

The PER data were subjected to the standard analysis of variance which examined the effects on PER values of four diets and nine chronological 3-day periods of time as shown in Table V.

From the computed results, the effect of diets on PER was very highly significant ($P < 0.01$) and the critical value as df 3/36 was $F_{0.01} = 4.38$. For time (periods), calculated F values were highly significant at 15.45 ($P < 0.01$) and the critical value at df 8/288 was $F_{0.01} = 2.58$. Also, the diets × time interaction was highly significant, where the calculated F value was 3.13 ($P < 0.01$) and the critical value at df 24/288 was $F_{0.01} = 1.86$. This indicates that the relationship between the different test diets × different 3-day test periods of time with respect to PER values was not consistently maintained for all diets during the 28-day assay period.

The mean liver weights corresponding to the different proteins, ratios to body weights, protein and fat contents of dried matter of livers (D.M.), and the ratio of protein to fat are shown in Table VI. Relative to body weight, livers of the two groups of animals which received SWP were heavier than the livers of those which received casein or ISP. The livers associated with casein were the largest and contained the most proteins and the least fat (on a dry weight basis). The livers associated with ISP were the smallest in relation to body weight and protein/fat ratio for dried matter. The lowest protein contents were those associated with SWP (on a dry basis) and the dry matter of livers associated with ISP–SWP contained the highest amount of fat. Since the liver is considered as an amino acid pool, any imbalance in the ratios of amino acids originating from various proteins in diets would affect the percentage of protein in analyzed livers.

The biological evaluation in this study indicates that SWP has significant nutritive value and improved protein quality by 74% when soybean protein in the diet was replaced by 50% of SWP. This will provide potential practical application if SWP is incorporated in canned or processed pet foods or used as an animal feed supplement for poultry and livestock. In addition, the use of SWP may help to eliminate water pollution as a result of using a large volume of water in shrimp processing plants.

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Received for review April 2, 1975. Accepted June 4, 1975.