Nutritive Quality of Wheat Flour and Bread Supplemented with Either Fish Protein Concentrate or Lysine[1]

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ABSTRACT

Studies were made to determine the relative supplemental value of fish protein concentrate (FPC) and of lysine when added to wheat flour. The nutritive quality of the supplemented wheat flour was determined before and after processing into bread. Mixtures were prepared containing wheat flour and either 0 to 25% FPC or 0.1 to 1.0% lysine. When unprocessed mixtures were added directly to rat diets at a 1.6% nitrogen level, 15% FPC and 0.2 to 0.4% lysine produced the maximum weight gain, protein efficiency ratio (PER), net protein utilization, and total protein and fat in the carcasses. Maximum responses obtained with FPC were greater than those obtained with lysine. When bread was added to diets at a 1.6% nitrogen level, 25% FPC and 0.4% lysine produced maximum weight gain and PER. The protein ratings for bread supplemented with 10 to 25% FPC were 35 to 150% higher than those for bread supplemented with lysine. When bread was included in diets on the basis of 80% by weight, the highest weight gains were produced with either 10% FPC or 0.4% lysine. With 10% FPC the weight gain was 36% higher than with 0.4% lysine.

The supply of dietary protein must be increased to meet the requirements of the world's increasing population. Proteins of either vegetable or animal origin can be used. Although vegetable proteins are less expensive and more abundant than animal, the quantity and quality of proteins from vegetable sources are usually inferior to those from animal sources.

One way that diets containing vegetable proteins can be improved is by supplementing them with either synthetic amino acids or protein concentrates. Laboratory studies have shown, for example, that the quality of protein in wheat flour can be improved by supplementing it with lysine (1,2,3). The addition of fish protein concentrate (FPC) to wheat flour will also increase the quality as well as the quantity of protein in the final mixture (4,5,6,7). However, no studies have been reported in which direct comparisons were made of the relative nutritional effectiveness of FPC and lysine when added to wheat flour at various levels.

For human consumption, wheat flour is usually processed into food products, such as bread. During processing, changes may occur that alter the nutritional characteristics. Accordingly, it is important to compare the supplemental value of lysine and FPC when they are added to wheat flour at various levels as well as when the mixtures are processed into bread. The present studies were therefore conducted to determine the relative supplemental value of FPC and lysine in improving the nutritional quality of the protein in wheat flour, both before and after processing it into bread.

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EXPERIMENT 1. SUPPLEMENTATION OF WHEAT FLOUR WITH EITHER FPC OR LYSINE

This study was conducted to determine the relative supplemental value of FPC and lysine in improving the nutritional quality of the protein from wheat flour.

Procedure

The nutritional quality was determined in a rat-feeding study, and gain in weight, protein efficiency ratio (PER), and net protein utilization (NPU) were used as criteria for evaluation.

The samples used in the study were: baker's grade wheat flour, L-lysine-hydrochloride, and FPC. The wheat flour and lysine were obtained from commercial sources, and the FPC was prepared from red hake (*Urophycis chuss*) by extraction with isopropyl alcohol (8). The wheat flour and FPC were analyzed for nitrogen, volatiles, and ash by AOAC methods (9). The amount of fat in the wheat flour was determined by ether extraction (9); the amount in FPC was determined by chloroform-methanol extraction (10).

Mixtures were prepared containing wheat flour and either FPC or lysine. FPC was used in the mixtures at levels of 0, 5, 10, 15, 20, or 25%. Lysine was used at levels of 0, 0.2, 0.4, 0.6, 0.8, or 1.0%.

Mixtures were added at a 1.6% nitrogen level to diets that were formulated to be isocaloric. Table I shows the composition of the basal diet. A diet containing 1.6% nitrogen from casein was included as a standard. In addition, a diet containing 0.6% nitrogen from whole-egg powder was used as a control in the determination of NPU (11).

Male weanling rats of the Holtzman strain were used to determine the nutritive value of the diets. The rats were received at 22 days of age and were fed a diet containing 15% casein for 2 days. They were then assigned to groups of ten on a weight basis, and the groups were randomly assigned to diets. The animals were fed *ad libitum* for 28 days, and the amounts of feed consumed and weight gained were recorded. PERs were calculated by dividing the amount of weight gained by the amount of crude protein consumed (nitrogen X 6.25). A small percentage of animals was affected by enzootic viral pneumonia. To minimize the influence of this factor, which was not related to the dietary treatments, we analyzed data from only the eight animals gaining the most weight in each group.

TABLE I. COMPOSITION OF BASAL DIET

	%
Nitrogen source[a]	
Dextrose[b]	80.0
Vitamin fortification mix[b]	1.0
Nonnutritive fiber[b]	5.0
Salt mixture USP XIV[b]	4.0
Corn oil[c]	10.0

[a] Dietary nitrogen in experimental diets was supplied by wheat flour supplemented with either FPC or lysine. In the standard diet, dietary nitrogen was supplied by ANRC casein, Sheffield Chemical, Norwich, N.Y.

[b] Obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

[c] Mazola corn oil, Corn Products Company, N.Y.

TABLE II. PROXIMATE COMPOSITION OF WHEAT FLOUR AND FISH PROTEIN CONCENTRATE

Component	Wheat Flour	Fish Protein Concentrate
Dry matter, %	86.9	94.9
Crude protein, %[a]	14.2	85.0
Ash, %	0.43	13.1
Fat, %	0.11	0.22

[a]Nitrogen X 6.25.

TABLE III. EFFECT OF SUPPLEMENTING WHEAT FLOUR WITH EITHER FPC OR LYSINE ON WEIGHT GAIN, PER, AND NPU

Dietary Protein Source		Average Daily Weight Gain g.	Protein Efficiency Ratio	Net Protein Utilization
Wheat flour %	FPC %			
100	0	0.90 ± 0.05[a]	1.04 ± 0.04	36.0 ± 1.8
95	5	3.17 ± 0.07	2.32 ± 0.04	49.2 ± 0.5
90	10	4.70 ± 0.17	2.97 ± 0.03	60.0 ± 1.2
85	15	5.79 ± 0.11	3.41 ± 0.05	66.9 ± 1.3
80	20	5.77 ± 0.27	3.35 ± 0.08	64.9 ± 1.4
75	25	5.43 ± 0.16	3.43 ± 0.05	68.5 ± 1.2
0	100	5.75 ± 0.18	3.51 ± 0.03	70.2 ± 1.3
Wheat flour %	Lysine %			
99.8	0.2	2.50 ± 0.11	2.10 ± 0.04	47.7 ± 1.1
99.6	0.4	2.39 ± 0.14	2.20 ± 0.03	52.8 ± 0.7
99.4	0.6	2.36 ± 0.10	2.19 ± 0.04	52.4 ± 1.2
99.2	0.8	2.20 ± 0.06	2.12 ± 0.04	51.3 ± 0.6
99.0	1.0	2.08 ± 0.06	2.08 ± 0.03	49.7 ± 0.3
Casein		4.85 ± 0.07	3.36 ± 0.05	68.3 ± 1.2
Tukey's W ($P < 0.05$)		0.65	0.22	9.8

[a]Standard error of the mean.

At the end of the 28-day period, all animals were killed and the carcasses of five representative animals from each group were analyzed individually. The five animals were selected on the basis of their numbered position in each group. The carcasses were prepared for analysis and sampled by the procedures of Hartsook and Hershberger (12). Total nitrogen was analyzed by the macro Kjeldahl procedure (9). Samples were dried in a vacuum oven for 16 hr. at 50°C. to determine the dry matter content. They were then analyzed for fat by extraction with ether for 16 hr. NPU values were calculated according to the method of Miller and Bender (11).

For each group, standard error of the means was calculated for data on weight gains, PERs, and NPUs. These data were also subjected to analysis of variance, and differences between means were determined by Tukey's procedure (13).

Results

Table II shows the proximate composition of the wheat flour and the FPC used in this study.

Table III shows the weight gains, PERs, and NPUs of animals consuming diets containing wheat flour supplemented with either FPC or lysine. Replacement of wheat flour with 5, 10, and 15% FPC produced major increases in weight gain, PER, and NPU. At the 15% level, the weight gain was more than six times and the PER value was more than three times that obtained with the unsupplemented diet. No further increases were found with supplements of 20 or 25% FPC. The weight gain produced with wheat flour supplemented with 10% FPC was similar to that produced with casein. With 15% FPC, the PER and NPU values were equivalent to those for casein.

Supplementing wheat flour with 0.2% lysine significantly improved weight gain, PER, and NPU values. No additional significant responses were found with higher levels of lysine, and the values tended to decline when from 0.6 to 1.0% lysine was used. The responses obtained for all levels of lysine were significantly lower than those obtained with casein.

The addition of 5% FPC or more to wheat flour produced higher weight gains than those obtained when lysine was used. The PER and NPU values obtained with 5% FPC were similar to those obtained with the various levels of lysine. Mixtures containing more than 5% FPC, however, produced higher PER and NPU values than the lysine-containing mixtures.

The relative difference between the NPU values for unsupplemented wheat flour and casein was considerably less than that between the PER values for unsupplemented wheat flour and casein. Also, when wheat flour was supplemented with either FPC or lysine, the response in NPU was less than that in PER. Campbell (14) reported that NPU is not sensitive to lysine deficient diets and that it overestimates the nutritive value of proteins that are deficient in lysine. Our results tend to agree with his observation.

Figure 1 shows total amounts of protein and fat in the animal carcasses. Addition of 5, 10, and 15% FPC to wheat flour greatly increased the total protein and fat contents. With 20 and 25% FPC, the total protein content was similar to that obtained with 15% FPC. However, the fat content in the carcasses decreased.

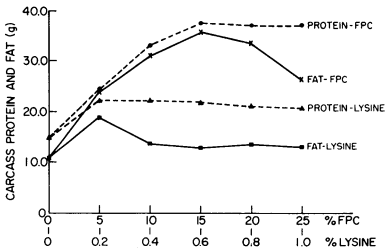

Fig. 1. Carcass composition of rats fed diets containing wheat flour supplemented with varying amounts of FPC or lysine.

Addition of 0.2% lysine to wheat flour also resulted in increases in carcass protein and fat. No further changes were found in the protein content with higher levels of lysine. The amount of carcass fat decreased, however, when the amount of lysine was increased from 0.2 to 0.4%. No further change in the amount of carcass fat was noted up to a level of 1.0% lysine.

As is evident in Fig. 1, greater amounts of both carcass protein and fat resulted when the animals consumed diets supplemented with FPC than when they consumed diets supplemented with lysine.

EXPERIMENT 2. SUPPLEMENTATION OF BREAD WITH EITHER FPC OR LYSINE

This study was conducted to determine the nutritional quality of bread that was prepared with wheat flour supplemented with either FPC or lysine.

Procedure

To determine the nutritional quality of the supplemented bread, we conducted an animal study. The bread was included in diets as the sole source of protein. The diets were fed to weanling rats, and the growth response of the animals and the efficiency of utilization of the protein were determined.

The wheat flour, FPC, and lysine samples were similar to those used in experiment 1. FPC was mixed with wheat flour at levels of 0, 5, 10, 15, 20, or 25%. Lysine was added to wheat flour at levels of 0, 0.1, 0.2, 0.4, 0.6, 0.8, or 1.0% of the final mixture. The mixtures were used to prepare bread according to the formulation given by Sidwell and Hammerle (15). The bread, after being baked, was freeze-dried and then ground through a Wiley mill. Samples of the bread were

TABLE IV. CRUDE PROTEIN AND LYSINE CONTENT OF BREAD SUPPLEMENTED WITH EITHER FPC OR LYSINE

Mixtures Added to Bread		Composition of Bread		
		Crude Protein[a]	Lysine	
		% of dry matter	% of crude protein	% of theoretical
Wheat flour %	FPC %			
100	0	16.0	1.97	97
95	5	19.6	3.32	97
90	10	23.2	4.35	100
85	15	27.4	5.09	101
80	20	31.7	5.36	97
75	25	34.5	6.06	102
Wheat flour %	Lysine %			
99.9	0.1	16.2	2.46	91
99.8	0.2	16.4	2.93	88
99.6	0.4	16.5	4.18	90
99.4	0.6	16.8	5.51	94
99.2	0.8	17.1	5.93	84
99.0	1.0	17.1	7.00	84

[a]Nitrogen X 6.25.

TABLE V. EFFECT OF SUPPLEMENTING BREAD WITH EITHER FPC OR LYSINE ON WEIGHT GAIN, PER, AND PROTEIN RATING

Mixtures Added to Bread		Average Daily Weight Gain g.	Protein Efficiency Ratio	Protein Rating[a]
Wheat flour %	FPC %			
100	0	1.13 ± 0.05[b]	1.13 ± 0.03	13.0
95	5	2.89 ± 0.12	2.04 ± 0.04	30.0
90	10	4.31 ± 0.14	2.53 ± 0.04	42.0
85	15	4.98 ± 0.13	2.86 ± 0.02	55.6
80	20	5.24 ± 0.31	3.04 ± 0.04	65.7
75	25	5.99 ± 0.05	3.35 ± 0.04	77.9
Wheat flour %	Lysine %			
99.9	0.1	1.49 ± 0.08	1.39 ± 0.03	16.6
99.8	0.2	2.59 ± 0.13	1.94 ± 0.05	22.9
99.6	0.4	3.87 ± 0.31	2.53 ± 0.07	31.0
99.4	0.6	2.93 ± 0.13	2.30 ± 0.07	28.6
99.2	0.8	2.55 ± 0.04	2.23 ± 0.03	26.0
99.0	1.0	2.42 ± 0.22	2.14 ± 0.10	26.5
Casein		5.34 ± 0.15	3.28 ± 0.03	...
Tukey's W ($P < 0.05$)		0.81	0.24	

[a]Calculated by multiplying the amount of protein in 150 g. of fresh bread by the PER (corrected to 2.5 for casein), as outlined by Campbell (16).

[b]Standard error of the mean.

analyzed for nitrogen by the macro Kjeldahl procedure (9), for lipids by ether extraction (9), and for lysine with an automatic amino acid analyzer.

The bread was incorporated into diets that were formulated in two different ways: either the diets were formulated to contain 1.6% nitrogen from the bread samples or the diets were formulated to contain 80% by weight of the bread samples. Both diets were formulated to be isocaloric. The composition of the basal diet was the same as that used previously.

The nutritive value of the diets was determined by the same procedures as were used in experiment 1. Weight gains were determined for all diets and PER values were calculated for diets containing 10% protein. Because a small percentage of animals in the experiment was affected by enzootic viral pneumonia, we analyzed data from the eight animals gaining the most weight in each group.

The protein ratings of the bread were determined by the method of Campbell (16). We calculated these ratings by multiplying the PER (corrected to 2.5 for casein) by the amount of protein in a "reasonable daily intake" (150 g. on a fresh basis) of bread.

Results

Table IV shows the crude protein and lysine contents of the bread supplemented with either FPC or lysine. As would be expected, addition of FPC to

bread greatly increased the concentration of protein, whereas the addition of lysine did not alter its concentration significantly. The actual amounts of lysine in the bread supplemented with FPC were similar to the theoretical values which were based on the amount of lysine in the components used in the bread formulations as determined by analysis. Bread supplemented with lysine, however, contained between 84 and 94% of the theoretical amounts that were used in the formulations. Thus, between 6 and 16% of the lysine was unaccounted for, and it was presumably lost during processing.

Table V shows the results of the animal study in which diets contained 1.6% nitrogen from the bread samples. Addition of mixtures of wheat flour and of from 5 to 25% FPC to bread resulted in increased gains in weight and PERs. The greatest percentage response was obtained when either 5 or 10% FPC was used. Supplements of from 0.1 to 0.4% lysine also increased both of these criteria. The PER results obtained with 10% FPC were similar to those obtained with 0.4% lysine. Whereas higher levels of FPC produced additional responses, greater amounts of lysine tended to depress the gain in weight and PER.

Table V also shows the protein ratings of the bread. The ratings increased as the amount of FPC was increased from 0 to 25%. The addition of 0.1, 0.2, and 0.4% lysine to bread also increased the ratings; however, they decreased when levels of lysine higher than 0.4% were used. The protein rating of bread supplemented with 5% FPC was similar to that of bread supplemented with 0.4% lysine.

Figure 2 shows the gains in weight of animals consuming diets with 80% bread. When 5 and 10% FPC were used, the weight gains increased substantially. The rate of weight gained was near maximum when 10% FPC was used, and only a slight increase occurred when higher levels of FPC were used. Gains in weight also increased when 0.1, 0.2, and 0.4% lysine were added to the wheat flour in bread. Higher levels of lysine, however, produced a slight decrease in the amount of weight gained. The results with 5% FPC were similar to those obtained with 0.4% lysine. However, higher levels of FPC produced gains in weight that were nearly 50% higher than the maximum amount obtained with lysine.

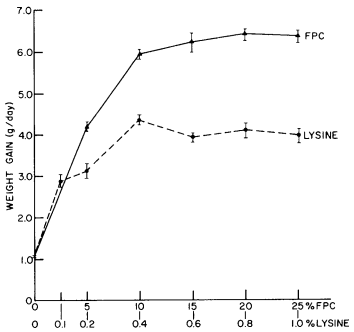

Fig. 2. Weight gains of rats fed diets containing 80% bread supplemented with varying amounts of either FPC or lysine. (Standard error of the means is shown at each point on curve.)

DISCUSSION

These studies were conducted to evaluate the nutritional effects of supplementing either wheat flour alone or wheat flour processed into bread with varying amounts of either FPC or lysine. The studies were designed to determine the optimum level of each supplement, as well as to compare the effectiveness of the two supplements. In each case, the amounts that were used covered a wide range, and it was expected that the higher levels would be above the amount needed to produce an optimum response. These wide ranges were used to obtain information on the effects of using FPC and lysine at levels below and above the optimum amounts.

In the first experiment in which wheat flour was supplemented with FPC, the optimum nutritional response was obtained with 15% FPC. Increases in weight gain, PER, NPU, and total protein in the carcass were obtained when 5, 10, and 15% FPC were used. Higher levels of FPC did not produce additional responses. Other workers have reported the results of studies in which wheat flour was supplemented with FPC (4,5,6,7), but they did not determine the minimum level of FPC needed to produce the maximum nutritional response.

In comparing results from different studies in which FPC is used as a supplement, particular attention should be given to the manner in which the levels of FPC are expressed. For example, Sure (6) studied the effect of supplementing wheat flour with FPC and expressed the amounts of FPC as percentages of the total diet. When either 1, 3, or 5% FPC was included in diets at the expense of wheat flour, substantial improvements were found in the gains in weight of rats and the PER. These improvements were the result of both an increase in the protein content of the diets and an increase in the quality of the protein.

In a study in which corn flour was enriched with FPC, Bressani (17) also expressed the amounts of FPC as percentages of the diet. He added increasing amounts of FPC to diets and concluded that a minimum of 3% FPC would produce the maximum PER, although 8% FPC was required to product the maximum gains in weight. As in the previous study, the responses obtained were the result of increases in both quantity and quality of the protein in the diet.

In our studies, the levels of FPC were expressed as percentages of wheat flour-FPC mixtures. Thus when the mixtures were incorporated into diets at a 10% level, the percentages of FPC in the diets were considerably less than in the original mixtures. For example, when the mixture containing 15% FPC was used, the amount of FPC in the total diet was 6%. In a study such as this, we believe that to express the amount of FPC as a percentage of the protein mixture is more meaningful than to express it as a percentage of the total diet.

The data obtained when wheat flour was supplemented with lysine indicated that the optimum level of lysine was about 0.2%. When this amount of lysine was added to wheat flour, significant increases occurred in the gain in weight, PER, NPU, and total carcass protein. When 0.4% lysine was used, an additional slight increase in PER and NPU occurred. However, higher levels of lysine had a depressing effect. The results obtained when wheat flour was supplemented with lysine are consistent with those reported by others (1,3).

Greater nutritional responses were obtained when wheat flour was supplemented with FPC than with lysine. This would be expected because FPC

supplied all the essential amino acids, whereas with lysine only the first limiting amino acid in wheat flour was supplied. Of particular interest, however, is the magnitude of the differences in nutritional quality. When wheat flour was supplemented with 15% FPC, the gains in weight of rats were nearly three times higher than those obtained with 0.2% lysine (Table III). Also, the highest PER and NPU values obtained with lysine were only 65 and 77%, respectively, as high as the maximum values obtained with FPC. Thus, supplementing wheat flour with FPC can result in a protein mixture that is superior in quality, as well as quantity, compared to that produced when only lysine is used. Hegsted (18) has recently questioned whether the quality of wheat flour supplemented with lysine would be high enough to fulfill the protein requirements of humans. Graham et al. (19), however, recently reported that the nutritional quality of wheat flour was significantly improved when supplemented with 0.2% lysine and fed to infants.

The second experiment was conducted to compare the effectiveness of FPC and lysine in improving the nutritional quality of bread. In evaluating protein supplements, we believe it is important that they be used in products for direct human consumption. Bread is a product that is widely consumed, so it is an excellent vehicle for supplying protein in the diet. Consequently, in this study, bread was the product that we used to evaluate the supplemental value of FPC and lysine.

Other studies have shown that the nutritional quality of bread can be improved by supplementing it with lysine (20,21,22,23). Jansen et al. (21) reported that part of the lysine added to bread was either lost or rendered unavailable during baking. In our studies, 6 to 16% of the lysine was lost during baking. However, the PER values obtained with lysine-supplemented bread were not lower than those obtained in experiment 1 with lysine-supplemented wheat flour. In fact, the PER of bread supplemented with 0.4% lysine was higher than that of unprocessed wheat flour supplemented with 0.4% lysine.

It has also been shown that the addition of FPC to bread will improve its nutritional quality (5,7,22). Morrison and Campbell (5) reported that the addition of 10% FPC to white bread increased the PER value by 198%. Yanez et al. (7) found that 6% FPC and 12% skimmilk powder produced similar increases in the protein values of bread. With both supplements, however, there was some loss in the quality of the protein during baking.

In our studies, we also found that processing reduced the quality of protein in bread supplemented with FPC. Using comparable levels of FPC, we found that the PER values for bread were lower than those for wheat flour. For example, with wheat flour, the highest nutritional value was obtained with 15% FPC, whereas with bread, a level of 25% FPC was required to product the highest nutritive value. Apparently lysine was not lost during baking (Table IV), so the decrease in nutritional quality may have been caused by a loss of other amino acids or a reduction in the availability of the amino acids.

Two important aspects to be considered when protein supplements are used in foods are their effect on the total protein in the product and their effect on the quality of the protein in the product. In the present study, addition of FPC to bread increased the amount of total protein, as well as the quality of the protein.

The use of lysine did not markedly affect the protein content, but the quality of the protein was significantly increased.

In an attempt to combine these two aspects into a single index, we used the method of Campbell (16) to calculate the protein rating. Although his method is empirical, it does provide a basis for comparing the over-all effects of using protein supplements in foods. When only the quality of the bread, as measured by PER, was determined, that with 10% FPC was found to be essentially equal to the sample with 0.4% lysine. However, when both quality and quantity of protein were considered by calculating the protein ratings, the value for bread with 10% FPC was 36% higher than the value for bread with 0.4% lysine. By this measurement, bread with 5% FPC was essentially identical to that with 0.4% lysine.

Another attempt was also made to evaluate the quality and quantity of protein in the bread samples by including them in diets on a basis of constant weight rather than on the basis of 10% protein. The responses obtained when the diets were fed to rats obviously reflected both the amount and the quality of protein in the bread. Because humans normally would not consume bread on an equal-protein basis, we believe that this method of evaluation is more practical.

When this method was used, animals consuming bread with 10% FPC gained nearly as much weight as those consuming bread with higher amounts of FPC. In the lysine series, bread supplemented with 0.4% lysine produced the highest gains in weight. In a comparison of the responses obtained between the two supplements, the differences were similar to those obtained when the protein ratings were calculated. That is, bread with 5% FPC and that with 0.4% lysine produced similar gains in weight. When 10% FPC was used, the gain in weight was 36% higher than when 0.4% lysine was used. Thus, by this method of evaluation, the optimum levels of supplementation were 10% for FPC and 0.4% for lysine.

Literature Cited

1. DESHPANDE, P. D., HARPER, A. E., and ELVEHJEM, C. A. Nutritional improvement of white flour with protein and amino acid supplements. J. Nutr. 62: 503 (1957).
2. HOWE, E. E., JANSEN, G. R., and ANSON, M. L. An approach toward the solution of the world food problem with special emphasis on protein supply. Am. J. Clin. Nutr. 20: 1134 (1967).
3. HOWE, E. E., JANSEN, G. R., and GILFILLAN, E. W. Amino acid supplementation of cereal grains as related to the world food supply. Am. J. Clin. Nutr. 16: 315 (1965).
4. METTA, V. C. Nutritional value of fish flour supplements. J. Am. Dietet. Assoc. 37: 234 (1960).
5. MORRISON, A. B., and CAMPBELL, J. A. Studies on the nutritional value of defatted fish flour. Can. J. Biochem. Physiol. 38: 467 (1960).
6. SURE, B. The addition of small amounts of defatted fish flour to whole yellow corn, whole wheat, whole and milled rye, grain sorghum and millet. I. Influence on growth and protein efficiency. II. Nutritive value of the minerals in fish flour. J. Nutr. 63: 409 (1957).
7. YANEZ, E., BARJA, I., MONCKEBERG, F., MACCIONI, A., and DONOSO, G. The fish protein concentrate story. 6. Quintero fish protein concentrate: protein quality and use in foods. Food Technol. 21: 1604 (1967).
8. BUREAU OF COMMERCIAL FISHERIES. Marine protein concentrate. Fishery Leaflet 584. U.S. Department of the Interior: Washington, D.C. (1966).

9. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis, (10th ed.). The Association: Washington, D.C. (1965).
10. SMITH, P., AMBROSE, M. E., and KNOBL, G. M. Improved rapid method for determining total lipids in fish meal. Com. Fisheries Rev. 26: 1 (1964).
11. MILLER, D. S., and BENDER, A. E. The determination of the net protein utilization of proteins by a shortened method. Brit. J. Nutr. 9: 382 (1955).
12. HARTSOOK, E. W., and HERSHBERGER, T. V. A simplified method for sampling small animal carcasses for analyses. Proc. Soc. Exptl. Biol. Med. 113: 973 (1963).
13. STEEL, R. G. D., and TORRIE, J. H. Principles and procedures of statistics. McGraw-Hill: New York (1960).
14. CAMPBELL, J. A. Methodology of protein evaluation: A critical appraisal of methods for evaluation of proteins in foods. WHO/FAO/UNICEF-PAG Nutrition Document R.10/Add.37 (1961).
15. SIDWELL, V. D., and HAMMERLE, O. A. Changes in physical and sensory characteristics of doughs and of bread containing various amounts of fish protein concentrate and lysine. Cereal Chem. 47: 739 (1970).
16. CAMPBELL, J. A. Evaluation of protein in foods for regulatory purposes. Agr. Food Chem. 8: 323 (1960).
17. BRESSANI, R. Enrichment of lime-treated corn flour with deodorized fish flour. In: Fish in nutrition. Fishing News Ltd. (London) (1962).
18. HEGSTED, D. M. Amino acid fortification and the protein problem. Am. J. Clin. Nutr. 21: 688 (1968).
19. GRAHAM, G. G., PLACKO, R. P., ACEVEDO, G., MORALES, E., and CORDANO, A. Lysine enrichment of wheat flour: Evaluation in infants . Am. J. Clin. Nutr. 22: 1459 (1969).
20. HARRIS, R. S., and BURRESS, D. A. Effect of level of protein feeding upon nutritional value of lysine-fortified bread. J. Nutr. 67: 549 (1959).
21. JANSEN, G. R., EHLE, S. R., and HAUSE, N. L. Studies of the nutritive loss of supplemental lysine in baking. I. Loss in a standard white bread containing 4% nonfat dry milk. Food Technol. 18: 367 (1964).
22. JANSEN, G. R., HUTCHINSON, C. F., and ZANETTI, M. E. Supplementation of bread with fish flour and amino acids–a comparison of evaluation methods. Food Technol. 20: 323 (1966).
23. ROSENBERG, H. R., and ROHDENBURG, E. L. The fortification of bread with lysine. II. The nutritional value of fortified bread. Arch. Biochem. Biophys. 37: 461 (1952).

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