feed contains 0.0125% DPPD (the level recommended for protection against encephalomalacia), only a 2-gram sample is needed for the test. Results obtained by use of this test are shown in Table I. Feeds stored as long as 4 weeks at 60° C. gave a test for DPPD if 0.0075% or more was originally added. Determination of carotene in alfalfa stored under the same conditions indicates that these accelerated storage conditions were equivalent to at least 6 months under normal conditions (25° C.).

Under specified conditions, the test appears specific for DPPD in feeds or alfalfa meal. Many materials were checked to determine whether they interfered in the test, including 16 amino acids, 11 amines and substituted amines, 9 vitamins, 8 drugs and 5 antibiotics used in feeds, 3 quinones, and miscellaneous feedstuffs.

A more sensitive test, but one not so specific for DPPD, is the following. Place 10 to 20 drops of the filtered hexane-acetone extract, as prepared in the foregoing test, in a small evaporating dish and evaporate nearly to dryness. Add 2 drops of concentrated nitric acid. The solution first turns blue and then red in presence of DPPD. A somewhat similar color is obtained in this test from 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Santoquin), but the acid-copper sulfate solution will not give a blue color with this chemical.

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Received for review March 26, 1955. Accepted June 4, 1955. Contribution 520, Department of Chemistry, Kansas Agricultural Experiment Station, Manhattan, Kan.

PROTEIN QUALITY AND SUPPLEMENTATION

Relative Nutritive Values of Proteins in Foods and Supplementary Value of Amino Acids in Pearled Barley and Peanut Flour

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A study was carried out on the relative nutritive values of the proteins in various foods at different levels of intake and during several periods of experimentation. The results are expressed as gains in body weight of the albino rat per gram of protein intake. Data are included on the influence of heat and duration of heating on the nutritive value of the proteins in dried nonfat milk solids (skim milk). Data are also recorded on the supplementary value of certain amino acids to the proteins in milled barley and peanut flour. Addition of 0.4% L-lysine, 0.5% DL-threonine, and 0.5% DL-methionine to the proteins in milled barley resulted in 151.1% increased growth and 224.7% increase in protein efficiency ratio. Supplementation of the proteins in peanut flour with 0.5% DL-methionine and 0.5% DL-threonine was followed by 60.6% gain in body weight and 61.5% increase in protein efficiency ratio.

There are essentially two methods of determining the nutritive value of proteins in foods. In 1909, Thomas (*20*) was the first to define the term biological value as the percentage of absorbed nitrogen which is retained by the body for the repair or synthesis of nitrogenous tissue. The measurement requires that the nitrogen intake, the nitrogen excretion, and the endogenous metabolic nitrogen be known. In 1924, Mitchell (*6*) applied this method to growing rats. Later, Mitchell and Beadles (*7*) emphasized the method of paired feeding and described the methods in detail. Osborne and Mendel (*8–10*) undertook an investigation of the methods of measuring quantitatively the comparative nutritive value of proteins, which they expressed as gains in body weight per gram of protein intake, defined as the protein efficiency ratio. Mitchell has critically reviewed the methods in use for determining the nutritive efficiency of proteins (*5*). Referring to the work of Osborne and Mendel, he says:

> As originally presented, the method involved a comparison of different proteins based on the maximum gains per gram of protein consumed. As ordinarily used by other investigators, however, no systematic attempt is made to find the maximum value. In some cases comparisons are made at one level of intake only, and in some cases comparisons of different proteins are made at different levels of intake, quite arbitrarily chosen. The values obtained with different proteins apparently stand in quite different ratios to one another, depending upon the level of protein intake (*6*).

In spite of such criticism, the comprehensive table by Block and Mitchell on the nutritive efficiency of the proteins in 41 foods (*1*) covers experimental periods of 4 to 8 weeks, and while they attempted to give data on protein contents of 10% such foods as rice, maize, and wheat flour were included. The maximum protein content in rations containing such foods could not be over 5, 7, and 8%, respectively; hence, the data submitted in this table do not represent accurate relative values of the protein

Table I. Protein Content of Various Foods

Food	Protein, %	Food	Protein, %
Whole wheat	14.7	Cottonseed meal	37.5
Whole rye	10.5	Peanut meal	47.0
Whole yellow corn	8.9	Peanut meal (OSE)[a]	47.9
Whole barley	12.4	Soybean meal	47.6
Pearled barley	10.0	Dried nonfat milk solids (low heat)	34.8
Rolled oats	14.9		
Grain sorghum	10.1	Dried nonfat milk solids (high heat)	35.1
Dried whole eggs	42.6		

[a] Organic solvent-extracted.

efficiency ratios of such foods. Because of such conflicting data in the literature, it was essential to reinvestigate the relative protein efficiencies of the cereal grains and some of the high-protein-containing foods by the Osborne and Mendel technique at different levels of intake during several periods of experimentation. However, the fact that in previous investigations frequent parallelisms were observed between per cent increase in food utilization and per cent increase in protein efficiency ratios and, in most cases, no parallelisms between biological values as determined by the nitrogen retention method and protein efficiency ratios, would indicate that the latter do not always express solely the gains in weight per gram of protein intake. The so-called protein efficiency ratios must also include gains in body weight produced by caloric intakes from the ration (*18*).

Experimental Procedure and Materials

This investigation was carried out on the Wistar strain albino rats. The foods used furnished the total proteins in the rations. Sufficient amounts of foods were incorporated in the rations to provide the desired levels of protein. The animals were 28 to 30 days old, when the experiments were started and weighed 50 to 54 grams each. The data given in Table I cover an experimental period of 10 weeks. There were 24 animals in each group, the sexes being equally divided. The rations contained 5 to 12% proteins; 4% of Sure's salt mixture No. 1 (*16*); 7% of hydrogenated vegetable shortening; 2% of cod liver oil; 1% of wheat germ oil; and the rest, percentagewise, glucose (Cerelose). The fat-soluble vitamins A, D, and E were supplied by the cod liver oil and wheat germ oil in the rations. All rations were supplemented with a liberal supply of the B vitamins separately from the rations (*12*). Folic acid was not added to the rations in this and the one on buckwheat following (*14*), because under the dietary regime followed this vitamin is synthesized in the intestinal tract of the rat. The role of vitamin B_{12} as a supplement to the proteins of plant origin has already been investigated (*13*) and hence was not considered in this and the work on buckwheat. Moreover, on the planes of protein intake used, no response to vitamin B_{12} was obtained as supplements to proteins of plant origin, unless deficient amounts of acids are also supplied (*19*). The animals were weighed once weekly and accurate records were kept of food consumption. From these data the protein efficiency ratios were determined, as expressed in gains in body weight per gram of protein intake.

The whole wheat and whole rye were furnished by General Mills, the whole yellow corn was supplied by A. E. Staley Manufacturing Co., and the whole barley, pearled barley, and rolled oats were furnished by the Quaker Oats Co. The cottonseed meal, peanut meal, and the soybean meal were commercial products, purchased in the open market. The grain sorghum was an Arkansas variety supplied by the Agronomy Department of the Arkansas Agricultural College. The dried whole eggs were purchased from C. A. Swanson & Sons, Omaha, Neb., and the dried nonfat milk solids were purchased from the Producers Creamery Co., Springfield, Mo.

Dried Nonfat Milk Solids The low-heat product is a dairy grade which involved heating to 163° F. for 16 seconds and the high-heat was a bakers' grade which was heated to a temperature of 205° F. for periods of about 30 minutes prior to drying. Table II shows that the higher temperature of drying for a longer period has resulted in appreciable reduction in the nutritive efficiency of the proteins in dried skim milk, particularly at the 5% level of intake—i.e., a 25.1% loss of protein utilization. These findings are in accord with observations reported in 1935 by Fairbanks and Mitchell (*2*) that the proteins in milk are sensitive to the intensities and duration of heat treatment employed in commercial drying. The results of this study are summarized in Tables I, II, III, and IV.

Discussion of Results

In Table II, at all levels of intake the proteins in dried whole eggs have the greatest nutritional efficiency, followed by those of dried nonfat milk solids (skim milk).

The high figures and differences in standard deviations and standard deviations of the means were due to differences in food intake, which resulted in individual differences in growth. The greater the increments of growth, the higher figures for such deviations were observed. However, such deviations were observed in all groups and the marked differences in growth and protein efficiency ratios were considerably greater among the groups on the various types of rations used than among individual animals in each group receiving different sources of proteins in foods.

Cereal Grains It is apparent that proteins in whole rye are superior in nutritive value to those in whole wheat at all levels of intake, which confirms the author's recent findings (*12*). In studying the relative nutritive values of the proteins in cereal grains, the period of experimentation is most important. For instance, Jones, Caldwell, and Widness (*4*), during a 6-week period of growth found for whole yellow corn at 4.5 and 7.5% levels of intake protein efficiency ratios 1.42 and 1.56, respectively. However, at 5% and 7% levels of intake, during a 10-week period of growth protein efficiency ratios in this whole cereal grain were found to be 0.50 and 0.91, respectively, and during a 6-week period these figures for 5% and 7% of protein intake were 0.82 and 1.22, respectively. The reason for the discrepancy between these figures and the figures of Jones and associates may result from different composition of rations, different grades of animals, and different varieties of corn. However, the influence of the period of experimentation on the protein efficiency ratios is apparent. Also, the transition from a 6- to a 10-week period of experimentation, in the case of whole yellow corn, has resulted in 50% mortality on the 5% protein level and a 25% mortality on the 7% protein intake. In Table II, the nutritive value of the proteins in rolled oats, used as a breakfast food in oatmeal, is high on all levels of protein intake. The high nutritive value is due to the fact that they are obtained by removing the fibrous hulls and adhering portions from the oat grain; hence rolled oats and oatmeal, like brown rice, are essentially whole-grain products from the nutritive standpoint (*3*). The proteins in whole barley at an 8% plane of intake compare in nutritive efficiency with those in peanut meal and are superior to those in whole wheat. Although the nutritive value of grain sorghum from the protein standpoint is low, lower than that in whole yellow corn, there was 100% survival on the 7% level of intake, whereas on the corn there was only 75% survival during the 10-week experimental period. Such findings indicate the necessity of longer periods of observation than 6 weeks for determining the nutritive value of proteins in foods of low biological value before conclusions are made on their efficiency for promoting growth.

Soybean, Cottonseed, and Peanut Meals At 10 and 8% levels of intake, the proteins in soybean meal have proved to be deficient in methionine. The addition of 0.5% DL-methionine to a ration containing 10% proteins derived from soybean meal resulted in trebling the body weight and in doubling the protein efficiency ratio. However, as little as 1.72 and 2.57% of defatted soybean flour have proved excellent supplements to the proteins in wheat flour and corn meal, respectively (*17*). In higher concentrations, introducing 25% proteins in rations, this product, supplemented with 0.1 to 0.3 γ of vitamin B_{12} per animal per day, produced excellent growth, reproduction, and lactation (*15*). The proteins in commercial cottonseed meal, peanut meal, and soybean meal are influenced largely by the method of processing. The highest values for proteins in cottonseed meal were found for organic solvent-extracted products and at 10% levels of intake considerable improvement in protein efficiency was observed following additions of 0.2% L-lysine and 0.2% DL-methionine (*11*).

Table III indicates that with all foods studied the protein efficiency ratios decrease with the increase of period of experimentation, because with increasing age the rate of growth decreases. It is then not surprising that variable figures are found in the literature, using the Osborne and Mendel technique (*8–10*), for determination of relative nutritive values of proteins in various foods, since many investigators use a 4- to 10-week period of growth (*6*).

Supplementary Value of Certain Amino Acids to Proteins in Pearled Barley and Peanut Flour. Table IV shows that supplementation of the proteins in pearled (milled) barley, fed at an 8% level, supplemented with 0.4% L-lysine, resulted in 57.2% increased growth and 50.0% increase in protein

Table II. Relative Nutritive Values of Proteins in Various Foods at Different Levels of Protein Intake

(24 animals in each group. Growth of 10 weeks. Average results per animal)

Type of Ration	Foods in Ration, %	Protein in Ration, %	Gains in Body Wt., G.	Total Food Intake, G.	Protein Intake, G.	Protein[a] Efficiency Ratio, %
Whole wheat	61.3	9.0	56.2 ± 5.7[b]	594.7	53.5	1.05 ± 0.05[c]
	54.5	8.0	43.8 ± 6.3	593.2	47.5	0.92 ± 0.04
	47.7	7.0	28.3 ± 4.5	522.8	36.6	0.77 ± 0.04
	34.1	5.0	19.8 ± 5.1	438.1	21.9	0.90 ± 0.08
Whole rye	85.7	9.0	78.2 ± 8.5	587.0	52.8	1.48 ± 0.04
	76.2	8.0	71.6 ± 6.9	638.7	51.1	1.40 ± 0.02
	66.7	7.0	83.6 ± 7.2	645.6	45.2	1.85 ± 0.04
	47.7	5.0	54.2 ± 6.0	556.8	27.8	1.95 ± 0.08
Whole yellow corn	78.5	7.0	30.7 ± 4.1	481.8	33.7	0.91 ± 0.06
	56.2	5.0	8.8 ± 2.9	354.0	17.7	0.50 ± 0.15
Grain sorghum	79.2	8.0	13.2 ± 3.1	388.0	31.0	0.43 ± 0.06
	69.3	7.0	6.1 ± 2.0	409.3	28.6	0.21 ± 0.04
Whole barley	64.5	8.0	65.1 ± 9.5	643.5	51.5	1.27 ± 0.04
	56.5	7.0	55.5 ± 8.2	668.1	46.8	1.19 ± 0.06
	40.4	5.0	14.4 ± 6.3	454.0	22.7	0.63 ± 0.10
Rolled oats	80.5	12.0	120.3 ± 12.1	617.7	74.1	1.62 ± 0.05
	67.2	10.0	112.7 ± 9.5	684.0	68.4	1.64 ± 0.04
	53.5	8.0	87.7 ± 4.6	657.7	52.6	1.67 ± 0.01
	33.6	7.0	66.0 ± 3.0	602.5	42.2	1.56 ± 0.04
Cottonseed meal	26.7	10.0	102.3 ± 11.8	797.2	79.7	1.28 ± 0.08
	21.4	8.0	66.7 ± 7.9	759.2	60.7	1.11 ± 0.10
Peanut meal	21.3	10.0	101.8 ± 11.0	934.2	93.4	1.09 ± 0.06
	17.0	8.0	79.0 ± 8.5	777.1	61.7	1.28 ± 0.04
Soybean meal	21.0	10.0	92.1 ± 7.6	729.6	73.0	1.26 ± 0.09
	16.9	8.0	52.3 ± 4.7	644.7	52.1	1.01 ± 0.13
Dried nonfat milk solids (low heat)	34.5	12.0	186.7 ± 14.1	896.0	117.5	1.59 ± 0.06
	28.7	10.0	164.1 ± 13.7	843.3	84.3	1.94 ± 0.07
	23.0	8.0	152.5 ± 11.8	851.9	68.1	2.23 ± 0.08
	20.1	7.0	132.8 ± 9.7	835.5	58.5	2.27 ± 0.07
	14.4	5.0	81.3 ± 6.9	716.1	35.8	2.27 ± 0.07
Dried nonfat milk solids (high heat)	34.2	12.0	168.8 ± 13.5	944.5	113.3	1.50 ± 0.06
	22.9	8.0	132.6 ± 11.9	866.7	69.4	1.97 ± 0.06
	20.0	7.0	113.8 ± 7.5	834.8	58.4	1.95 ± 0.05
	14.3	5.0	59.2 ± 5.8	707.7	35.4	1.67 ± 0.06
Dried whole eggs	28.2	12.0	144.6 ± 10.6	664.6	79.7	1.81 ± 0.06
	23.1	10.0	157.3 ± 11.5	669.0	66.9	2.35 ± 0.09
	18.8	8.0	145.0 ± 9.9	715.2	57.2	2.53 ± 0.08
	11.6	5.0	87.9 ± 5.7	668.9	33.4	2.60 ± 0.07

[a] Expressed as gains in body weight per gram of protein intake.
[b] Standard deviation
[c] Standard deviation of the means

Table III. Influence of Period of Experimentation on Protein Efficiency Ratios of Various Foods

Foods in Rations	*Period of Experimentation, Weeks*	*Protein Efficiency Ratio*[a]
Dried whole eggs	4	3.40 ± 0.13[b]
	6	3.04 ± 0.13
	8	2.65 ± 0.09
	10	2.35 ± 0.09
Dried nonfat milk solids (low heat)	4	2.67 ± 0.07
	6	2.34 ± 0.06
	8	2.04 ± 0.06
	10	1.94 ± 0.07
Soybean meal	4	1.46 ± 0.10
	6	1.45 ± 0.09
	8	1.45 ± 0.05
	10	1.26 ± 0.09
Peanut meal	4	1.40 ± 0.08
	6	1.33 ± 0.09
	8	1.17 ± 0.05
	10	1.09 ± 0.06
Cottonseed meal	4	1.46 ± 0.06
	6	1.32 ± 0.06
	8	1.29 ± 0.06
	10	1.28 ± 0.08
Rolled oats	4	2.07 ± 0.02
	6	1.92 ± 0.01
	8	1.73 ± 0.02
	10	1.64 ± 0.04

[a] Expressed as gains in body weight per gram of protein intake.
[b] Standard deviation of means.

efficiency ratio. The further addition of 0.5% DL-threonine was followed by 78.6% additional gain in body weight and 118.4% further increase in protein efficiency. The supplementation of pearled barley with L-lysine, DL-threonine, and 0.5% DL-methionine resulted in 15.3% additional growth and 56.3% increase in protein utilization. The reason that greater gains were made in protein efficiency than in increased growth following addition of DL-threonine and DL-methionine, in presence of L-lysine, is that greater gains in body weight were produced on less food intake.

Table IV also shows that the nutritive value of the proteins in organic solvent-extracted peanut flour can be improved considerably by the addition of 0.5% DL-methionine and 0.5% DL-threonine to rations containing 9% of proteins in this product. The addition of these amino acids was accompanied by 60.6% increased growth and 61.5% increase in protein efficiency ratio.

Acknowledgment

The author wishes to acknowledge the technical assistance given by Leslie Easterling, Joy Dowell, and Mary Crudup. The author wishes to express his appreciation to E. I. du Pont de Nemours & Co. for the generous supply of L-lysine, and to the Dow Chemical Co. for the liberal supply of DL-methionine.

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Received for review February 14, 1955. Accepted June 30, 1955. Published with the approval of the Director, Arkansas Agricultural Experiment Station. Aided by grants of E. I. du Pont de Nemours & Co., and The National Institutes of Health.

Table IV. Supplementary Value of Certain Amino Acids to Proteins in Pearled Barley and Peanut Flour[a]

(24 animals in each group. Growth of 10 weeks. Average results per animal)

			Gains in Body Weight				Protein Efficiency Ratio[b]	
Type of Ration	*% in Ration*	*Protein in Ration, %*	*G.*	*Increase, %*	*Total Food Intake, G.*	*Protein Intake, G.*		*Increase, %*
Pearled barley (P.B.)	80.0	8.0	56.8 ± 7.6[c]		546.1	43.7	1.30 ± 0.07[d]	
P.B. + 0.4% L-lysine	80.0	8.0	89.3 ± 8.3	57.2	572.7	45.8	1.95 ± 0.07	50.0
P.B. + 0.4% L-lysine + 0.5% DL-threonine	80.0	8.0	133.9 ± 10.1	135.8	480.0	38.4	3.49 ± 0.09	168.4
P.B. + 0.4% L-lysine + 0.5% DL-threonine + 0.5% DL-methionine	80.0	8.0	142.7 ± 10.3	151.1	423.0	33.8	4.22 ± 0.13	224.7
Peanut flour (P.F.)	16.7	9.0	62.2 ± 6.8		636.4	57.3	1.09 ± 0.06	
P.F. + 0.5% DL-methionine	16.7	9.0	89.6 ± 7.8	44.1	635.2	57.2	1.57 ± 0.03	44.0
P.F. + 0.5% DL-methionine + 0.5% DL-threonine	16.7	9.0	99.9 ± 8.2	60.6	630.9	56.8	1.76 ± 0.05	61.5

[a] Organic solvent-extracted.
[b] Expressed as gains in body weight per gram of protein intake.
[c] Standard deviation.
[d] Standard deviation of the means.