require the use of separate strips, or a modified technique for the analysis of protein in the strips which would cover a wide range of protein concentration.

A number of papers on ionophoresis of milk and whey proteins by Schulte and Müller (*8*) which appeared recently, although not bearing directly on the quantitative determination of these proteins, are of interest in connection with the results reported in this paper.

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QUALITY OF PLANT PROTEINS

Nutritive Value of Quinua Proteins

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Quinua (*Chenopodium quinoa,* Willd), an Incan seed, used in human nutrition, supplies protein of high quality as judged by growth and lipotropic responses, in white weanling rats. Under experimental conditions, at least seven essential amino acids had to be added to produce slightly better weight gains than that given by 90% quinua alone. Combinations of quinua and casein, giving the same total dietary protein as that supplied by quinua alone did not support better growth than quinua, confirming the findings of other workers. However, the addition of 3% casein to quinua, giving a total dietary protein 12.42%, produced an excellent growth of 36.7 grams per rat per week and a normal liver fat.

THE PROTEIN VALUE OF MANY FOODS is often higher in combination with other proteins, at various intake levels, than when fed alone. Hart (*11*) strongly warned against condemning a food because its proteins, alone, are not of high biological value—e.g., barley, rye, corn, and wheat. He also emphasized the supplemental value of skim milk and other animal proteins. Sure (*21*) reported recently on an experiment which showed that the proteins in buckwheat flour at an 8% plane of intake are the best source of high biological value proteins in the plant kingdom. The abnormal deposition of fat in the liver is one sign of protein malnutrition and is related particularly to the activity of the lipotropic amino acids (*2, 4, 6, 9–11, 14, 16, 18–20*). Children fed on plant proteins lacking a good balance of amino acids develop fatty livers (*18*).

White and coworkers (*22*) have shown with both young rats (74 grams, fed for 54 days) and depleted adult rats (218 to 230 grams, 14 days repletion) that, at equal levels of protein intake (6 or 9%), the proteins of quinua produced gains equal to or superior to those of milk protein (skim, whole, dried), and that milk protein supplementation to quinua did not produce better gains than quinua alone.

Deshpande and associates (*6*) supplemented white rice proteins with 0.2% L-lysine and 0.24% DL-threonine and obtained gains of 19.1 to 22.0 grams per week per rat, but the liver was fatty, 27.6 to 33.9% on a dry weight basis. Rice alone at 87% in the diet gave a poor rate of growth which was about 7 grams per week and the liver was also fatty, 30.7% fat. Lysine, 0.4%, and threonine, 0.5%, were needed to obtain a liver lipide content of 10.6 to 12.6%, which is considered normal. However, when the high level of these amino acids was used, the growth was reduced, 16 to 17.5 grams. This depression was overcome by adding the other essential amino acids in amounts equivalent to the levels supplied by 3% casein. The liver fat was normal, 13.6%, and a maximum growth of about 27 grams per week per rat was obtained with this supplementation.

The amino acid composition of quinua protein as reported by White and coworkers (*22*) shows a good balance of amino acids. Therefore, the quinua proteins were tested for growth and lipotropic function, as a measure of nutritive value (*2, 16*), and also to obtain more information on the supplemental effect of casein and of the essential amino acids contained in 3% casein.

Experimental

Weanling male rats (Hol zman Co.), averaging 47 to 54 grams, were separated according to weight into similar groups of six, and housed individually in screen-bottomed cages. The rats were fed *ad libitum* for 2 weeks, weighed weekly, and their feed consumption records were kept. The composition of the basal diet is given in Table I. All supplements were made at the expense of sucrose. Two drops of halibut liver oil fortified with vitamins E and K (*10*) were administered orally once a week.

The quinua was the white sweet variety obtained from the Agricultural Experiment Station of Puno, Peru. The samples were "saponin-free" (sourless) and the dry seeds were finely ground. The nitrogen of all proteins and amino acids was determined by the Kjeldahl method using mercuric oxide as a catalyst. All diets were isocaloric (*5*), about 366 calories per 100 grams, and Solka Floc replaced sucrose to balance the calories (Experiments A and

Table I. Diet Composition

Basal, %		Vitamin Mixture, G.	
Quinua[a]	87.00	Calcium pantothenate	2.0
Sucrose	3.60	Niacin	2.5
Choline chloride	0.15	Riboflavin	0.5
Salts IV (*13*)	4.00	Thiamine	0.5
Vitamins mixture	0.25	Pyridoxine	0.25
Corn oil	5.00	Inositol	10.00
		Biotin	0.01
		Folic acid	0.02
		B_{12}	0.002
		Ascorbic acid	5.0
		Sucrose to make up 250 grams	

[a] Washed and unwashed quinua were employed.

B). The casein was extracted for three 2-hour periods with boiling alcohol.

After 2 weeks, each rat was killed by a blow on the head and decapitated. The liver was excised and the lipide determination was made following the method outlined by Hawk and Elvehjem (*12*).

Results

The average values for growth, liver fat content, and protein intake are presented in Tables II and III.

In the first and second experiments (Table II), quinua alone gave as good growth as a combination of two thirds of the protein from quinua and one third from casein. On the other hand, the addition of 3% casein to 87% quinua, giving a protein content of 12.42%, produced a significant increase in growth (Experiment B.) This level of protein cannot be reached with quinua alone, since it contains 11.25% or 10.93% protein, washed or unwashed quinua, respectively. Both types of quinua supported similar good growth responses, although a few rats had less preference for the unwashed seed.

In Experiment C, all groups showed less growth than in trial B, but again 3 or 5% casein added to 87 or 85% quinua produced a significant improvement. Even gelatin, which is considered a nutritionally incomplete protein, increased body gain; a similar effect was shown with rice in the work done by Deshpande and associates (*6*). Analytical data (*21*) indicate that valine, methionine, and phenylalanine may be limiting in quinua. However, when these amino acids were added to 87% quinua (Experiment C, 4) there was no change in the rate of growth. At the start of the second week, half of this group received 1% urea as an extra source of nitrogen, but no growth response resulted.

The data presented in Table III (Experiment D) show that the addition of tryptophan, leucine, or isoleucine to 87% quinua, as indicated, did not produce body gains higher than that obtained with 90% quinua alone. Again a supplement of 3% casein supported a growth of 36.7 grams per rat per week.

In the fifth experiment (Table III), eight essential amino acids increased the weight gains and no further response could be secured by adding 1.5% ammonium citrate. Although the rats fed 87% quinua plus lysine and threonine grew better than the animals receiving the grain alone, there was no additional response to histidine or valine in presence of lysine and threonine. Leucine and isoleucine improved the growth slightly, which was unchanged by adding tryptophan.

Experiment F was carried out to check trial E. Both agreed very well and explained the individual variations in growth responses (Experiment F, 2, compare identical groups on Tables II and III). Methionine in combination with valine and phenylalanine was ineffective (Experiment C, 4); however, in the presence of lysine, threonine, valine, histidine, leucine, and isoleucine this amino acid seems to be slightly beneficial (Experiment F, 8). The growth effect of phenylalanine, in mixtures of amino acids as indicated in Experiment G, remains to be elucidated, as the control groups were missing.

In preliminary experiments of longer duration, a mixture of 87% unwashed quinua and 3% fish meal (11.9% protein) supported an average growth of 33 or 27 grams per rat per week, during 5 or 10 weeks of trial, respectively. Whereas the rats fed a combination of 87% unwashed quinua, 2% casein, and 1% gelatin, which supplied a total protein content of 12.2%, gained an average weight of 37.6 or 36.4 grams per rat per week during 4 or 5 weeks of experiment, respectively.

The liver fat was normal in all the experimental groups, which also indicates the high quality of protein in quinua. There was close correlation between growth responses and protein intake.

Discussion

This study confirms reports that quinua has a high quality of protein and shows that plant proteins can maintain a normal liver fat in association with a comparatively good growth response (Table IV). This emphasizes the good balance of amino acids present and may explain why the responses were not very significant even when eight essential amino acids were added to quinua.

Table II. Nutritive Value of Quinua Proteins, Growth and Lipotropic Responses, and Animal Protein Supplementation

(Weanling male rats fed *ad libitum* on isocaloric diets for 2 weeks)

Expt.	Quinua, %	Supplement	Protein[a] Content, %	Growth/Rat/Wk., G.	Liver Fat, Dry Weight, %	Protein Intake/Rat/Wk., G.
A 1	90		10.13	19.1 ± 3.7[b]	12.1 ± 0.6[b]	6.1
2	60	3% casein	9.39	17.3 ± 2.4	12.7 ± 0.4	5.5
B 1	90		10.13	24.4 ± 3.7	11.3 ± 0.5	6.8
2	60	3.4% casein	9.75	23.9 ± 2.0	11.4 ± 0.7	7.1
3	87	3% casein	12.42	35.1 ± 3.3	10.2 ± 0.5	10.0
4	90	(unwashed)	9.84	22.2 ± 2.0	11.3 ± 0.4	6.9
C 1	87	3% casein	12.42	28.8 ± 2.0	10.5[d]	8.7
2	85	5% casein	14.18	32.9 ± 1.7	11.5	10.2
3	87	3% gelatin	12.85	26.5 ± 2.8	12.6	9.4
4	87	VM Ph[c] (urea)	10.28	18.3 ± 5.0	11.4	6.6

[a] Protein, N% × 6.25.
[b] Values are mean ± standard error of mean for group of 6 rats.
[c] V, 0.46% DL-valine; M, 0.1% DL-methionine; Ph, 0.2% DL-phenylalanine.
[d] Pooled sample for each group was prepared by taking 1 gram from liver of each rat.

Table III. Nutritive Value of Quinua Proteins, Growth and Lipotropic Responses, and Amino Acids[a] and Animal Protein Supplementation

(Weanling male rats fed *ad libitum* on isocaloric diets for 2 weeks)

Expt.	Quinua, %	Supplement	Protein Content, %	Growth/Rat/Wk., G.	Liver Fat, Dry Weight, %	Protein Intake/Rat/Wk., G.
D 1	90		10.13	23.8 ± 5.6[b]	12.6[c]	7.2
2	87	3% casein	12.42	36.7 ± 2.6	11.4	10.0
3	87	Tr	9.82	22.3 ± 4.2	11.3	7.0
4	87	LiTr	10.30	22.1 ± 3.3	11.5	7.1
5	87	LI	10.26	22.3 ± 3.7	11.9	7.4
E 1	87		9.78	18.3 ± 2.8	14.4	5.6
2	87	3% casein	12.42	27.1 ± 3.2	12.2	7.6
3	87	8AA	11.23	27.2 ± 4.6	11.6	7.1
4	87	8AA + 1.5% Am. c.[d]	12.39	28.0 ± 2.0	12.0	7.9
5	87	LyT	10.13	22.5 ± 2.2	11.7	6.3
6	87	LyTH	10.31	21.9 ± 3.2	11.5	5.9
7	87	LyTV	10.47	18.7 ± 3.3	10.0	5.3
8	87	LyTVH	10.65	20.4 ± 2.4	10.8	5.7
9	87	LyTVHLI	11.13	24.6 ± 3.7	10.9	7.1
10	87	LyTVHLITr.	11.17	24.3 ± 3.7	10.3	6.9
F 1	87		9.78	18.8 ± 2.2	13.4	6.0
2	87	3% casein	12.42	35.2 ± 2.4	12.2	9.0
3	87	3% gelatin	12.85	25.8 ± 2.0	13.9	9.6
4	87	8AA	11.23	27.8 ± 1.0	11.7	6.1
5	87	LyT	10.13	21.4 ± 2.6	12.0	6.3
6	87	LyTVH	10.65	20.8 ± 2.0	11.0	6.4
7	87	LyTVHLI	11.13	23.0 ± 1.4	11.5	7.6
8	87	LyTVHLIM	11.19	26.6 ± 3.9	11.8	6.2
G 1	87	Unwashed	9.51	15.0 ± 1.4	12.7	4.7
2	87	8AA + Ph	11.06	28.4 ± 1.4	10.8	7.1
3	87	LyTLIM + Ph	10.51	22.9 ± 1.7	10.7	6.1
4	87	3% casein	12.15	29.5 ± 3.2	11.3	8.6
5	86.6	4% casein	13.04	30.2 ± 3.3	11.0	8.7

[a] Amino acids added were ≅ 3% casein—e.g., 8AA + Ph means in percentages: 0.2 L-lysine·HCl, Ly; 0.24 DL-threonine, T; 0.14 L-histidine·HCl, H; 0.46 DL-valine, V; 0.3 L-leucine, L; 0.44 DL-isoleucine, I; 0.05 DL-tryptophan, Tr; 0.1 DL-methionine, M; and 0.2 DL-phenylalanine, Ph.
[b] Values are mean ± standard error of mean for group of 6 rats.
[c] Pooled sample for each group was prepared by taking 1 gram from liver of each rat.
[d] Dibasic ammonium citrate.

Quinua alone at 90% level gave body gains above those obtained when lysine and threonine were added and such gains were not much below those obtained when eight essential amino acids were fed in mixtures containing even much higher amounts of protein.

Tryptophan appeared to be slightly less important than methionine, when each was in combination with at least seven amino acids. This supports the suggestion that there is little likelihood of a total sulfur–amino acid deficiency in quinua (*22*). The results with gelatin (which lacks tryptophan) further suggest that this amino acid is present in sufficient quantity and is readily available in quinua.

As the highest level of quinua fed supplies only 10.13% protein, optimum growth response is not expected. However, the response is much better than that obtained with any of the cereals. For example (Table IV), wheat supplies almost as much protein as washed quinua, but the growth with wheat is about one half that obtained with quinua. Even soybean protein does not allow maximum growth in the rat because of the low level of cystine and methionine. Quinua has a higher level of methionine than soybean (*3*, *22*), therefore, its supplementary value is evident (*1*, *7*, *14*, *22*). This report shows also that mixtures of quinua plus small amounts of a suitable animal protein, making up about 12% protein in the diet, afford a well balanced amino acid pattern, which can promote excellent results in growth and lipotropic activity as well.

Rose and associates (*17*), Lardy and Feldott (*15*), and Frost and Sandy (*8*), for example, found that diammonium citrate, glutamic acid, glycine, urea, and ammonium acetate spared the indispensable amino acid nitrogen by affording extra nitrogen for synthesis of the nonessentials. The authors fed urea and diammonium citrate plus intact protein in quinua (10 to 11%) without any response.

Table IV. Quality of Some Plant Proteins

Levels in Diet, %	Protein, %	Average Gain/Rat/Wk., G.	Liver Fat, Dry Wt., %
90 Quinua, washed	10.13	24.4 ± 3.7	11.3 ± 0.5
90 White rice (*9*)	5.90	8.8 ± 0.6	31.8 ± 2.6
90 Corn (*9*)	7.40	2.2 ± 0.9	12.8 ± 1.8
88 Wheat (*9*)	9.10	9.9 ± 1.2	11.8 ± 1.2
87 Quinua, washed	9.78	18.8 ± 2.2	13.4

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PROTEIN ASSAY

Assessing Protein Quality with the Individual Protein-Depleted Chick

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Techniques and equipment are described for carrying out protein assays on individual protein-depleted chicks. Assays have been carried out at dietary levels of 6.5, 9, and 12% of protein using a mixture of casein, gelatin, and DL-methionine as a standard of reference. Dairy products have been assayed as the sole source of protein and in combination with other proteins commonly used in experimental chick rations. Data are presented to show the differences in response of protein-depleted chicks and rats to three sources of protein.

In studying poultry nutrition and in evaluating poultry feeds and ingredients, the accepted procedure is to carry several chicks in one pen. Individual weights can be secured, but food consumption must be taken on the pen as a whole. The chick's requirement for unusually high temperatures during the first few days of life makes the battery brooder a necessity and has probably been the reason for the practice of group feeding throughout the entire test period. In determining amino acid requirements and evaluating protein quality in chicks, the group-feeding practice is also followed. The method of Grau and Almquist (*4, 5*) is widely used. Chicks are carried for 2 weeks on a good growing ration and are then distributed according to weight into groups for the 8-day assay period. The test protein is fed at a dietary level of 20% and the results are expressed in terms of per cent gain per day.

The rat has long been used to evaluate the nutritive value of protein and it seemed that some of the techniques might be applied to the chick. The weight regeneration method of Cannon (*3, 10*) appeared to be adaptable as work could be done on an older animal, and in the case of the chick, the period when extra heat is required would be past. The chick could be conveniently housed alone and feed and protein consumption data of individual chicks could be obtained.

Procedure

Day-old female New Hampshire chicks are reared for 10 days in battery brooders on a good growing mash. They are then placed in individual rat cages of the type shown in Figure 1. Light for the lower tiers is supplied by 15- or 25-watt light bulbs. The cages are held slightly forward over the pans to catch any food thrown out by the chicks. The pans are filled with sawdust and a strip of paper is placed over the sawdust under the food cups to facilitate recovery of spilled food. The food cup is secured in the rear of the cage with

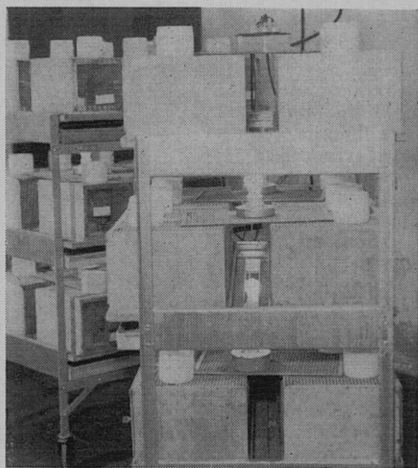
Figure 1. Cage stands for individual housing