STUDIES ON THE NUTRITIVE VALUE OF PLANT PROTEINS: PART I.

PULSE PROTEINS—THEIR IMPROVEMENT BY AMINO ACID SUPPLEMENTATION *

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INTRODUCTION

Studies on the investigations regarding the protein contents of seven important pulses, their digestibility, biological and growth-promoting values under raw and autoclaved conditions (Esh and Som, 1952) and some of their essential amino acid compositions (Esh and Som, 1953, 1954) have been reported in previous communications. These studies along with others (Ratnaprabha and Magar, 1953; Vijayaraghavan and Srinivasan, 1953) tend to indicate that some of these pulse proteins are not only associated with trypsic inhibitors affecting their nutritive value but all of them are deficient in methionine and/or tryptophan when compared to a standard protein (say, egg protein). Significant growth responses were, therefore, observed when these pulses were autoclaved and supplemented with methionine and fed to growing rats as an exclusive source of protein (Esh and Som, 1952; Ratnaprabha and Magar, 1953). Finding that such growth responses are still sub-optimum, attempts were made to increase further if possible the biologic utilization of these proteins by supplementing with other essential amino acids.

Two of the pulses, namely Bengal gram and lentil, which are deficient in sulphur containing amino acids and tryptophan, were taken for this purpose. Since the limiting amino acids as suggested by Mitchell and Block (1946) are likely to improve the protein utilization, the pulses were first studied after supplementing with methionine and/or tryptophan. In view of the observations that the utilization of rice protein (Pecora and Hundley, 1951), wheat protein (Sure, 1952) and groundnut protein (Esh and Basu, 1954) is significantly increased when supplemented with threonine indicating that the amino acids which are 'limiting' chemically may not be always so physiologically, experiments were conducted also after supplementing these pulses with threonine.

EXPERIMENTAL

The pulses Bengal gram and lentil obtained from the market and ground to 40 mesh were used after autoclaving at 15 lbs. pressure for 30 minutes in all these experiments. The protein efficiency of these pulses (gain per gram of protein eaten) was measured by the rat growth method by feeding weanling litter mate rats diets containing equal suboptimal amounts of proteins (12%) provided by the respective pulses. The basal diet contained in grams, hydrogenated fat 9, salt mixture (U.S.P. XIV) 4, cod-liver oil 2, choline chloride 0·2, sufficient autoclaved pulse powder to maintain 12% protein level and corn starch to make 100 grams. 100 grams of this diet was supplemented with 1 c.c. vitamin B mixture † and 0·5 c.c. of vitamin

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* Based on the paper read at the Symposium on 'Proteins in health, disease and industry' held in August, 1954, under the auspices of the National Institute of Sciences of India.

† Thiamine hydrochloride 20 mg., riboflavin 30 mg., nicotinic acid 200 mg., pyridoxine hydrochloride 12·5 mg., panthenol 150 mg., folic acid 5·0 mg. and biotin 0·5 mg. dissolved in 50 c.c. alcohol.

K and E mixture.* The essential amino acid contents of both the pulses as analysed by chemical and microbiological methods (methionine, tryptophan, lysine, threonine and phenylalanine chemically and leucine, isoleucine and valine microbiologically) have been tabulated in Table I, with those of casein and egg protein as collected from Block and Bolling (1945).

TABLE I

Essential amino acid composition of Bengal gram and lentil.
(Calculated on the basis of 16% nitrogen)

Amino Acid	Egg	Bengal gram	Lentil	Casein
Methionine ..	4·1	1·2	0·6	3·5
Tryptophan ..	1·5	0·75	0·6	1·2
Lysine ..	7·2	6·3	5·1	7·9
Threonine ..	4·9	2·8	3·0	4·1
Phenylalanine ..	6·3	4·9	3·98	5·6
Leucine ..	9·2	8·2	5·50	9·9
Isoleucine ..	8·0	6·5	5·80	6·5
Valine ..	7·3	5·5	5·1	6·7
Arginine ..	6·4	6·9†	6·9†	4·2
Histidine ..	3·0	2·3†	2·1†	2·1

The approximate amounts of the essential amino acids supplied by the pulses at the level used (12%) in these experiments are placed in Table II. In this table also is shown the order of deficiency of the essential amino acids in pulses as calculated from their amino acid composition and the minimum daily amino acid requirements for the growing rats as reported by Rose (1937). For comparative

TABLE II

The order of deficiency of the essential amino acids in pulses when compared with daily requirement for growth

Amino acids	Requirement for growth % of diet	Bengal gram		Lentil		Casein	
		Supplied in the diet %	Deviation from requirement %	Supplied in the diet %	Deviation from growth %	Supplied in the diet %	Deviation from growth %
Methionine ..	0·6	0·14	−76	0·072	−88	0·42	−30
Tryptophan ..	0·2	0·09	−55	0·072	−64	0·15	−25
Lysine ..	1·0	0·76	−24	0·61	−39	0·95	−5
Threonine ..	0·5	0·33	−34	0·36	−28	0·5	0
Histidine ..	0·4	0·25	−37	0·25	−37	0·25	−37
Phenylalanine ..	0·7	0·59	−15	0·48	−30	0·67	−3
Valine ..	0·7	0·66	−6	0·60	−14	0·79	+12
Leucine ..	0·8	0·98	+22	0·66	−17	1·18	+47
Isoleucine ..	0·5	0·78	+56	0·69	+38	0·78	+56
Arginine ..	0·2	0·82	+300	0·82	+300	0·5	+150

* Menadion (vitamin K) 20 mg. and alpha-tocopherol 1,000 mg. dissolved in 100 c.c. arachis oil.

† Calculated from the results reported by Vijayaraghavan and Srinivasan (1953).

studies, therefore, different groups of rats were fed experimental pulse diets after supplementation with most deficient amino acids making approximate allowance for the inactive forms. The slight variation in total nitrogen contents of the diets due to such supplementation was, however, compensated by varying the pulse and starch contents. Weanling albino rats as raised in our own colony and weighing about 40–45 gms. were divided among the groups with usual precautions as to distribution of litters, sex and weight. They were housed individually in screen bottom metal cages with food and distilled water *ad libitum*. Groups receiving a standard diet containing casein in the same level were included in each set of experiments as a guide for establishing relative rates of growth. All these tests were continued for three weeks. Animals were weighed twice in a week and total food intake of each animal was recorded.

A second set of experiments was designed with enzymic digests of these pulses both before and after supplementation with deficient amino acid. The pulses were digested with papain and the protein hydrolysates thus obtained were fed to protein depleted adult rats as the sole source of protein in order to find the nature of protein regeneration during the repletion phase particularly after supplementation with deficient amino acids.

Results and Discussion

The results of feeding Bengal gram diet at 12% protein level both with and without amino acid supplementation are presented in Table III. As previously obtained (Esh and Som, 1952), methionine supplementation made significant growth increment over that obtained with the pulse only. It is interesting to find that single supplementation with other deficient amino acids like tryptophan, threonine or lysine could not induce greater growth. But when supplemented with these amino acids in presence of methionine, only threonine could enhance significant growth response beyond that obtained after supplementation with methionine

Table III

Average growth increments in rats when Bengal gram diets were fed for 3 weeks after supplementation with deficient amino acids. (Average of six rats used in each group)

Diet	Wt. gain in gm.	Gain increase %	Food intake in gm.	Protein intake in gm.	Protein efficiency ratio
Bengal gram	24·1 ± 0·6*	..	155·3	18·6	1·29
,, ,, +methionine ..	32·3 ± 0·8	34·1	160·7	19·3	1·67
,, ,, +tryptophan ..	22·0 ± 1·5	0	152·0	18·2	1·21
,, ,, +threonine ..	24·8 ± 1·7	0	148·2	17·8	1·39
,, ,, +lysine ..	24·0 ± 1·2	0	155·0	18·5	1·30
,, ,, +methionine and tryptophan.	35·1 ± 2·1	45·6	156·4	18·8	1·87
,, ,, +methionine and threonine.	44·5 ± 1·5	84·6	162·0	19·4	2·29
,, ,, +methionine and lysine.	32·8 ± 0·9	36·0	156·0	18·7	1·22
,, ,, +methionine, threonine and tryptophan.	48·3 ± 1·2	100·4	172·1	20·7	2·34
,, ,, +methionine, threonine, tryptophan and lysine.	47·9 ± 2·1	100·0	170·0	20·4	2·35
Casein	50·5 ± 1·8	109·5	178·6	21·4	2·36

* Standard error of the mean calculated by the formula $\sqrt{\frac{\Sigma(x-\bar{x})^2}{n(n-1)}}$.

only. The slight increment with tryptophan in presence of methionine is of doubtful significance although in presence of methionine and threonine it could induce a little greater growth activity. The addition of lysine with the group methionine, threonine and tryptophan could not enhance further growth. The rats of all the comparable groups have taken significant quantity of food and the data for protein efficiency ratio as calculated tend to indicate that the efficiency of protein utilization has significantly increased when the diet was supplemented with methionine and threonine. Apparently two conclusions may be arrived at that while the supplementation with the most limiting amino acid methionine can improve the nutritive value of the pulse protein, other deficient amino acids fail to do so in absence of methionine but threonine in presence of methionine can accelerate growth activity of pulse protein. Actually how these added amino acids function cannot be explained at this stage of our knowledge.

Similar experiments have been conducted with lentil protein and the results are presented in Table IV. In this case also maximum growth increment was obtained when the diet was supplemented with the most deficient amino acid—methionine and supplementation with other deficient amino acids has practically no effect.

TABLE IV

Growth increments in rats when lentil diets (at 12% protein level) were fed for 3 weeks after supplementation with deficient amino acids. (Average of 6 rats used in each group.)

Diet	Wt. gain in gm.	Gain increase %	Food intake in gm.	Protein intake in gm.	Protein efficiency ratio
Lentil diet	12 ± 1·1 *	..	149·5	18·0	0·67
,, + methionine	33·25 ± 1·8	177	161·5	19·38	1·72
,, + tryptophan	11·9 ± 1·9	..	148·0	17·8	0·67
,, + lysine	12·1 ± 1·8	..	150·5	18·1	0·67
,, + threonine	11·7 ± 2·1	..	151·0	18·12	0·64
,, + methionine and tryptophan.	37·5 ± 1·8	212	153·3	18·4	2·04
,, + methionine and threonine.	36·2 ± 2·1	202	173·3	20·8	1·74
,, + methionine and lysine	32·8 ± 1·1	175	158·0	18·96	1·71
,, + methionine, tryptophan and threonine.	50·2 ± 2·5	318	161·0	19·34	2·59
,, + methionine, tryptophan, threonine and lysine.	49·07 ± 2·2	309	156·7	18·81	2·60

* Standard error of the mean calculated by the formula $\sqrt{\frac{\Sigma(x-\bar{x})^2}{n(n-1)}}$.

In presence of methionine, however, while addition of either tryptophan or threonine has induced almost equal growth stimulation (in case of tryptophan a little greater) curiously enough, their incorporation together has influenced the growth activity to a significantly higher level raising the protein efficiency ratio from 0·67 (pulse only) to 2·59. Addition of the next deficient amino acid lysine failed to improve the nutritive value further.

The results obtained with enzymic digest of lentil when fed to protein depleted rats are placed in the Graph I, below.

The nature and degree of growth increments observed during the repletion phase when the different diets were fed to the protein depleted rats tend to indicate the same trend of results when the intact pulse was fed after supplementation with the deficient amino acids.

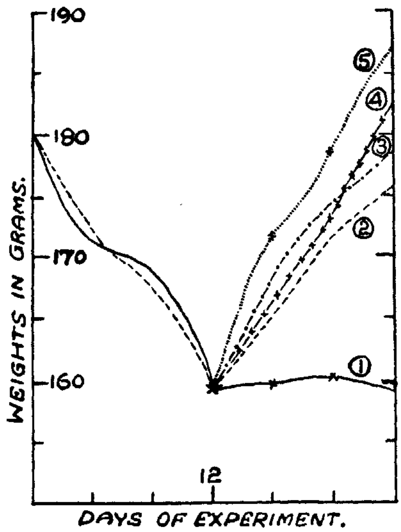

FIG. 1. Weight regeneration of protein-depleted adult rats when an enzymic digest of lentil (5% protein solution) was fed as an exclusive source of protein for 12 days. (1) Lentil hydrolysate, (2) 1 with methionine, (3) 1 with methionine and tryptophan, (4) 1 with methionine, tryptophan and threonine, and (5) casein hydrolysate.

The results as reported above tend to show that although supplementation with most deficient amino acid, methionine, increases the growth-promoting capacity of the pulses significantly, additional growth was obtained when tryptophan and threonine were added with methionine. Thus with Bengal gram, methionine has raised the protein efficiency ratio from 1·29 to 1·67 and methionine along with tryptophan and threonine the same has been raised to 2·34, about 100% improvement over the basal diet. With lentil also while methionine has enhanced the protein efficiency ratio from 0·67 to 1·7, addition of threonine and tryptophan has raised the same further to 2·6 about 50% improvement over the value obtained by methionine supplementation only. The similar results obtained with enzymic digest of lentil after amino acid supplementation give testimony to the fact that the same pattern of amino acids as obtained from the pulses and as modified by the addition of deficient amino acids is effective in promoting growth as well as in regenerating weight loss when fed to the protein-deficient animals.

Further work is in progress regarding the influence of supplementing the pulse proteins with other protein foods rich in methionine, threonine and tryptophan on their growth-promoting value.

SUMMARY

Improvement on the nutritive value of pulses, Bengal gram and lentil was effected by supplementing them with deficient amino acids—methionine, tryptophan and threonine. It has been observed that when these pulses were fed at 12% protein level, supplementation with most deficient amino acid methionine significantly enhanced their growth-promoting capacity. Supplementation with other deficient amino acids like tryptophan, lysine or threonine individually in absence of methionine was ineffective, but in presence of methionine, tryptophan and/or threonine significantly enhanced the nutritive value further. Thus maximum improvement was obtained with the combination of methionine, tryptophan and threonine raising the protein efficiency ratio of Bengal gram from 1·29 to 2·34 that of lentil from 0·67 to 2·59.

The same trend of improvement of the pulses was observed when an enzymic digest of the pulse lentil was fed to protein-depleted adult rats for regeneration of growth.

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