Biological response of rats fed amino acid supplemented pea bean *(PHASEOLUS VULGARIS)* diets[1]

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SUMMARY

Sprague-Dawley immature male white rats were fed a 10% level (1.6% nitrogen) isocaloric defatted whole dried egg control diet and four pea bean (Phaseolus vulgaris) test diets without amino acid supplementation or supplemented with either 0.3% DL methionine, 0.1% DL tryptophan, or both, for 28 days. Analysis of mean differences indicates that methionine supplementation was effective in increasing consumpton and weight gains.

Tryptophan supplementation reduced fecal nitrogen excretion, but greatly increased urea nitrogen and total urine nitrogen excretion. The results indicate that tryptophan supplementation did not increase nitrogen utilization. Excess free amino acids, not used in metabolism, were proportionately excreted as amino nitrogen.

INTRODUCTION

McPherson (1) suggested that one of the first steps needed to improve low-quality plant protein foods might be the addition of prescribed amounts of amino acids, such as methionine and lysine.

Inexpensive plant proteins such as the cereal grains and the legumes lack certain amino acids. All the common cereal grains are deficient in lysine while the legumes are primarily

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deficient in methionine. Adding proper amounts of lysine to cereals and of methionine to plant foods enhances the protein efficiency of these two plant foods. Further protein efficiency might be attained by adding small amounts of other amino acids (2).

Several investigators (3, 4, 5, 6, 7, 8) have shown that the lack of methionine was the principal growth-limiting factor, with the exception of pigeon peas (5), of rats fed low-protein legume diets.

Based on amino acid analysis of dried whole egg and pea beans (*Phaseolus vulgaris*) (9) a study was undertaken to determine if the addition of DL methionine and DL tryptophan to a pea bean legume protein might be more effective in promoting growth and nitrogen utilization of growing male rats than DL-tryptophan or DL-methionine alone. Changes in weight gains, nitrogen retention and utilization were determined to clarify the nutritional value of a legume protein supplemented with more than one amino acid.

EXPERIMENTAL

Groups of three male rats (Sprague-Dawley strain), each weighing 103 to 129 grams, were kept in individual stainless steel metabolic cages and fed quantities (10-18 grams) of isocaloric, isonitrogenous reference and test diets (table 1) for 28 days. The quantities of racemic amino acid (0.3% DL-methionine and 0.1% DL-tryptophan) supplements were based on the findings of Salmon (10), and Sauberlich *et al.* (11).

Commercially dried whole egg was fat-extracted with hexane (boiling point 65° to 75° C) in a Soxhlet apparatus for 36 hours. The dried defatted whole egg was used as the reference diet based on the report of Sumner *et al.* (12).

Pea beans, autoclaved for 30 minutes at 15 pounds pressure to decrease toxicity and increase palatibility, were then dried at room temperature and ground to a fine flour (13, 8).

A 10% level of protein (1.6% nitrogen) was chosen on the basis of information reported in the literature (2, 5). Since most vegetables have a low protein content, incorporating more than 10% of plant protein in the diet could make it impossible to include enough of the other dietary constituents.

TABLE 1

COMPOSITION OF EXPERIMENTAL DIETS

DIETARY CONSTITUENTS	DIETS				
	Egg g %	Pea Beans g %	Pea Beans+ Methionine g %	Pea Beans+ Tryptophan g %	Pea Beans+ Methionine+ Tryptophan g %
Defatted dried whole egg[1]	14.8				
Pea bean[2]		40.4	40.4	40.4	40.4
Corn oil	5.0	5.0	5.0	5.0	5.0
Vitamin mix[3]	2.2	2.2	2.2	2.2	2.2
Wesson salt mixture[4]	4.0	4.0	4.0	4.0	4.0
Cerelose[5]	72.0	48.4	48.1	48.3	48.0
Alphacel	2.0				
DL methionine[3]			0.3		0.3
DL tryptophan[3]				0.1	0.1
Total protein[6]	10.4	10.5	10.5	10.5	10.0
Per cent nitrogen	1.66	1.68	1.68	1.68	1.60
Calories/g[7] (direct calorimetry)	3.80	3.84	3.83	3.85	3.88

1 Fat extracted in the laboratory by hexane. Henningsen, Inc. Springfield, Missouri, U.S.A.
2 Michelite pea bean (*Phaseolus vulgaris*).
3 Vitamin Diet Fortification Mixture in Dextrose and synthetic amino acids purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.
4 Wesson Salt mixture W is formulated per Wesson modification of Osborne and Mendels salt mixture (Science, 75: 339, 1932).
5 Cerelose Corn Products Refining Co., Argo, Illinois, U.S.A.
6 N × 6.25 — analyzed in laboratory.
7 Calories/g — analized in laboratory.

Portions of the defatted dried whole egg and pea bean flour were analyzed for amino acid content by Dr. Wayne Ryan[1], University of Nebraska College of Medicine (Table 9) after the experimental work was completed.

All laboratory rats were weighed individually at a specified time each week. Food was weighed daily and each rat was offered a total of 401 grams of food during the experimental period. All uneaten food from each rat was collected and weighed daily to determine the amount of food actually consumed.

Seven-day composites of pooled feces from each rat were dried in a vacuum oven at 10 pounds pressure to a constant weight and then ground, re-weighed and stored in individual bottles. Urine was collected daily in flasks containing approximately 5 ml of 10% sulfuric acid, as described by Rose *et al.* (14). Seven-day composites of pooled urine samples for each rat were stored in a refrigerator until analyzed.

Total nitrogen was determined for the protein foods, experimental diets, urine and feces by the micro-Kjeldahl procedure (15). Urine samples (1 cc) were adjusted to pH 7.6 with 0.2 N NaOH and analyzed for total urea (16). For the amino nitrogen determination (17) 25-cc urine samples adjusted to pH 7.6 with 1 N NaOH were analyzed.

Caloric content of diet samples was estimated by direct combustion in the Parr oxygen calorimeter.

RESULTS AND DISCUSSION

Results were analyzed by a series of analyses of variance, all of which showed highly significant treatment effects. Designed studies of these treatment effects were made by t tests of differences between treatment means and are reported in

1 Dr. Ryan (personal communication) describes his method as follows: "The samples were weighed and dried to constant weight at 100° to determine per cent moisture. A weighed sample was placed in a glass ampoule and made 6 N with hydrochloric acid. The ampoules were sealed, placed in a dry ice-alcohol bath and attached to a high vacuum pump. At 50 microns, the ampoules were sealed and placed in a constant temperature heating block at 100° C for 24 hours. After lyophilization to remove the hydrochloric acid, the residue was dissolved in pH 2.2, 0.2 N sodium citrate buffer and chromatographed on a Spinco 120B amino acid analyzer (accelerated system with micro-cuvette). The tryptophan content was determined on a separate sample by the Spies and Chambers method. Cystine and methionine were determined by the method of Moore, using performic acid oxidation to methionine sulfone and cysteic acid."

Tables 2 through 8. Table 2 shows that pea bean and pea bean plus tryptophan diets are eesentially the same in terms of weight gain. Thus the addition of tryptophan was of no value. The other three diets, whole defatted egg, pea bean plus methionine, and pea bean with both methionine and tryptophan, resulted in considerably greater weight gains. Adding

TABLE 2

COMPARISON OF DIFFERENCES IN MEAN WEEKLY WEIGHT GAINS OF THREE RATS PER GRAM OF NITROGEN RETAINED

DIET	grams/ week	PBMT	PBT	PBM	PB
		22.7	7.8	21.2	8.3
Egg	22.5	.2	14.7***	1.3	14.2***
PB[1]	8.3	14.4***	.5	12.9***	
PBM[2]	21.2	1.5	13.4***		
PBT[3]	7.8	14.9***			

*** Significant at .001 level of probability, others not significantly different.

1 Pea Beans

2 Pea Beans + Methionine

3 Pea Beans + Tryptophan

TABLE 3

COMPARISON OF DIFFERENCES IN MEAN WEEKLY GAINS (grams) PER GRAM OF FOOD CONSUMED BY THREE RATS

DIET	grams/ week	PBMT	PBT	PBM	PB
		.26	.10	.29	.11
Egg	.31	.05	.21**	.02	.20**
PB	.11	.15**	.01	.18**	
PBM	.29	.03	.19**		
PBT	.10	.16**			

** Significant at the .01 level.

both tryptophan and methionine to pea bean did not result in weight gains greater than pea bean with methionine. Once again indicating that tryptophan was of no value. The rats consumed more than 99 per cent of the food provided for three diets: whole defatted egg, pea bean with methionine, and pea bean with both methionine and tryptophan. They consumed 91.2 per cent of pea bean with tryptophan and only 76 per cent of unsupplemented pea bean diet. Differences in nitrogen retention among the pea bean diets were significant in themselves, but not in terms of retention per gram of food consumed. However, all were lower than the egg diet. Since consumption of food accounted for differences in nitrogen retention among supplemented pea bean diets, it is of interest to consider the effect of consumption on weight gains. Table 3 shows this relationship and indicates that additions of tryptophan did not increase utilization of pea bean diets as did methionine. Table 4 shows differences in nitrogen intake which was of course due to differences in consumption.

Excretion of fecal nitrogen is shown in Table 5 in terms of food consumed. There was less fecal nitrogen excretion with egg diet than with any pea bean diet. The higher level of unabsorbed nitrogen from a legume protein diet was reported by other investigators (3, 18). Addition of tryptophan lowered fecal nitrogen excretion within the pea bean diets. Supplementing pea bean diets with tryptophan increased the

TABLE 4

COMPARISON OF DIFFERENCES IN MEAN WEEKLY NITROGEN INTAKE (in milligrams) IN THREE RATS

DIET	mg N	PBMT	PBT	PBM	PB
		1600	1533	1677	1274
Egg	1660	60**	127***	17	386
PB	1274	326***	259***	403***	
PBM	1677	77**	144***		
PBT	1533	67**			

*** Significant at .001 level of probability.
** Significant at .01 level of probability.
* Significant at .05 level of probability.

TABLE 5

COMPARISON OF MEAN WEEKLY MILLIGRAMS OF FECAL NITROGEN PER GRAM OF FOOD CONSUMED FOR THREE WHITE RATS

DIET	mg N	PBMT	PBT	PBM	PB
		3.587	3.305	3.842	3.869
Egg	1.285	2.302***	2.557***	2.020***	2.584***
PB	3.869	.282	.564***	.027	
PBM	3.842	.537**	.255		
PBT	3.305	.282			
PBMT	3.587				

** Significant at the .01 level.
*** Significant at the .001 level.

TABLE 6

COMPARISON OF DIFFERENCES IN MEAN WEEKLY EXCRETION OF NITROGEN (in milligrams) IN URINE BY THREE RATS

DIET	mg N	PBMT	PBT	PBM	PB
		93	158	106	86
Egg	101	8	57**	5	15
PB	86	7	72**	20	
PBM	106	13	52**		
PBT	158	65**			

** *Significant at the .01 level.*

amount of nitrogen excreted in the urine (Table 6). The milligrams of urine nitrogen per gram of food consumed is 1.73 for pea bean with added tryptophan. This is significantly higher than the other pea bean diets that ranged from .93, 1.06, to 1.13 or whole defatted egg 1.01. Urea nitrogen was significantly higher with tryptophan added than for all other diets (Table 7). This relationship is not changed in considering

TABLE 7

COMPARISÓN OF DIFFERENCES IN MEAN WEEKLY EXCRETION OF UREA NITROGEN (In milligrams) IN URINE OF THREE RATS

DIET	mg N urea N	PBMT	PBT	PBM	PB
		19	26	17	16
Egg	15	4	11**	2	1
PB	16	3	10**	1	
PBM	17	2	9**		
PBT	26	7*			

** Significant at .01 level.
* Significant at .05 level.

food consumption. The higher urea with the tryptophan diets does not account for all of the higher urine nitrogen. These results indicate that addition of methionine results in better utilization of pea bean diet. The addition of tryptophan to pea bean diet results in some decrease in fecal nitrogen but the main effect is depressed nitrogen utilization with the excess being released in the urine.

TABLE 8

COMPARISON OF DIFFERENCES IN MEAN WEEKLY EXCRETION OF AMINO NITROGEN (per milligram) FOR THREE RATS

DIET	mg N amino N	PBMT	PBT	PBM	PB
		27	15	17	7
Egg	12	15***	3	5*	5*
PB	7	20***	8*	10**	
PBM	17	10**	2		
PBT	15	12***			

* Significant at .05 level.
** Significant at .01 level.
*** Significant at .001 level.

TABLE 9

COMPARISON OF AMINO ACIDS IN EDIBLE PORTION OF THE FOODS AND REQUIREMENTS FOR RAT GROWTH

Essential Amino Acids for Rat Growth [1]	Proportions [1] required for Rat Growth	Dried Whole Egg [2] Average g per g total Nitrogen	Pea Bean [2] (Phaseolus vulgaris) Average g per g total Nitrogen	Dried Whole Egg [3] Defatted mg/100.mg dry wt.	Pea Bean [3] (Phaseolus vulgaris) mg/100.mg dry wt.
Lysine	5	0.400	0.464	4.3	1.5
Leucine	4	0.550	0.537	6.3	2.2
Valine	3.5	0.464	0.379	4.8	1.4
Phenylalanine	3.5	0.361	0.345	4.2	1.5
Methionine	3	0.196	0.063	4.3	0.62
Cystine		0.146	0.062	4.2	0.61
Isoleucine	2.5	0.415	0.355	4.0	1.3
Threonine	2.5	0.311	0.271	3.6	1.2
Histidine	2	0.150	0.178	1.5	0.5
Arginine	1	0.410	0.376	4.0	0.5
Tryptophan	1	0.103	0.058	0.42	0.96
Glycine	0	0.221	0.106	2.6	0.9
Aspartic Acid	0	0.438	0.419	8.1	3.3
Serine	0	0.525	0.325	5.5	1.5
Proline	0	0.265	0.269	3.0	1.1
Glutamic Acid	0	0.773	1.000	10.2	4.0
Alanine	0		0.356	4.3	1.1
Tyrosine	0	0.269	0.241	2.7	0.6

[4] Rose's Requirements (19).

[2] Home Economics Report No. 4 (9).

[3] Dr. Wayne Ryan. University of Nebraska, College of Medicine: Lincoln, Nebraska.

Diets with amino acids added were all significantly higher in total amino nitrogen recovered than the pea bean diet (Table 8). The whole egg diet was not greatly different from the pea bean diet. While the diets with DL-tryptophan and DL-methionine were different from pea bean alone, they were not different from each other or the defatted whole dried egg diet. The diet containing both DL-methionine and DL-tryptophan was the highest in total amino nitrogen excreted and this was significantly higher than all other diets. This would indicate that free amino acids in the diet, not used in metabolism, are excreted as amino nitrogen, and that increasing free amino acid content of diet over amount utilized will increase the amino nitrogen excreted proportionately. This phenomenon of mean differences in metabolic utilization of free amino acids is closely related to growth requirements of a young rat (Table 9). Actual analysis of the amino acids of our test food did not agree with the Home Economics Report No. 4 (9) especially for tryptophan (Table 9).

Based on the Home Economics Report No. 4 we added DL-tryptophan to our pea bean diet as their analysis shows deficiencies in methionine and tryptophan. After our laboratory results were studied, an amino acid analysis was performed on our test foods, to clarify any differences which could effect the results. Comparisons indicated (Table 9) that a mere inspection of the amino acid pattern of a protein food will not afford a sound basis for judgment of their nutritive value or even for determining which amino acids limit their nutritive value. Final judgment always lies with an actual amino acid analysis followed by controlled feeding trials.

RESUMEN

Respuesta biológica de ratas alimentadas con dietas elaboradas a base de frijoles (Phaseolus vulgaris) y suplementadas con aminoácidos

Ratas machos de 103-129 gr. de peso se alimentaron con cantidades pesadas de raciones isocalóricas y con 10% de proteínas preparadas a base de huevo desgrasado o de frijoles (Phaseolus vulgaris) con o sin suplementación con 0.3% DL-methionina ó 0.1% DL-triptofano.

Se observó que la adición de metionina aumenta grandemente el consumo y la retención de N y el aumento de peso. DL-triptofano afectó la excreción total de N y de urea. La excreción de N amínico era mayor en las raciones adicionadas con aminoácidos que con las dietas de huevo o frijoles solamente.

Los resultados indican que una suplementación excesiva de una ración de frijoles con DL-triptofano resultó en una utilización reducida del nitrógeno y que un exceso de aminoácidos libres no utilizados metabólicamente fueron excretados proporcionalmente en la orina y detectados como amino-nitrógeno. Un exceso de DL-triptofano apareció en la orina principalmente en forma de urea en contraste con DL-metionina.

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