THE TRYPTIC INHIBITOR AND THE AVAILABILITY OF CYSTINE AND METHIONINE IN RAW AND GERMINATED SOYA BEANS

by

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INTRODUCTION

In the course of their studies on the effect of germination on the nutritive value of soya bean protein, EVERSON *et al.*[7] showed that, on germination, there was a marked increase in the growth promoting value of the protein of soya bean. While discussing their results, they pointed out that this increase in nutritive value could not be explained by the mere increase in the digestibility of the protein. At about the same time, BLOCK AND BOLLING[2] analysed raw and germinated soya bean proteins for certain essential amino acid contents. They could not detect any difference in the tyrosine, phenylalanine, tryptophane, cystine and methionine contents of the proteins. Hence the possibility that the increase in nutritive value was due to a change in the amino acid composition of the protein during germination was also ruled out.

ALMQUIST *et al.*[1], HAYWARD *et al.*[9], and EVANS *et al.*[6] have shown that methionine is the limiting amino acid in the nutritive value of soya bean protein. The results of JOHNSON *et al.*[12] also showed that the retention of absorbed sulphur was greater in the case of autoclaved soya bean protein than in the case of the raw unheated protein. These results have been confirmed recently by EVANS *et al.*[6], who studied the availability of cystine and methionine of soya bean protein on chicks. There was a greater availability of methionine in the case of the autoclaved protein.

On the basis of these results, it was therefore thought desirable, to study the availability of the sulphur amino acids of germinated soya bean protein compared with raw bean. It was hoped that this experiment would throw some light on the cause for the higher growth-promoting value of germinated soya bean protein.

Since the metabolism of the sulphur amino acids is closely parallelled by the metabolism of protein, in addition to the sulphur balance studies, nitrogen balance studies were also carried out simultaneously. The conditions under which the experiments were carried out and the experimental technique are outlined below.

The germinated soya bean used in the following experiments was prepared by germinating a good quality of soya bean for 72 hours, drying it in the sun and then powdering it so as to pass through a 40 mesh sieve.

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EXPERIMENTAL

The experiment was carried out on a group of six healthy adult rats. The bean proteins were fed to the animals at a 10% level the composition of the diets being, protein 10%, fat 10%, salt mixture (sulphur free) 4%, vitaminized starch 5%, cane sugar 10%, and the rest made of starch and other carbohydrates contained in soya bean. After feeding the germinated protein diet for 8 days during which the urine and faeces of the animals were collected, the animals were given a period of rest for four days on the laboratory stock diet. The animals were then given the raw soya bean protein diet for 8 days, the excretions being collected as usual during the period. After giving rest for four days, the animals were kept on the usual nitrogen free diet for one week, the excretions of the animals being collected in the last four days only.

The urine and faeces of the animals were collected daily and preserved in the refrigerator below 4° C. Since sulphuric acid could not be used as a preservative, toluene was used for preserving the urine samples. The pooled urine sample for each animal at the end of each experimental period was filtered, made up to volume, and aliquots were taken for analysis of nitrogen, total sulphur, inorganic sulphur, ethereal sulphur, cystine sulphur and methionine sulphur. The faecal samples were moistened with 2% copper acetate to prevent loss of sulphur while drying (as suggested by CUTHBERTSON *et al.*[3]), dried at 95° C, weighed, powdered and aliquots were taken for analysis of nitrogen and total sulphur. The excretions of the animals on the nitrogen free diet, were analysed for nitrogen only and the average daily endogenous nitrogen excretion values for the urine and faeces were determined.

The diets fed to the animals were also analysed for nitrogen and sulphur.

Nitrogen was determined by the KJELDAHL micro-method. Total sulphur in the urine was determined by FOLIN's method[8] using the modified BENEDICT reagent as an oxidizing mixture. Inorganic sulphur and ethereal sulphur were determined by the method of FOLIN. Cystine sulphur and methionine sulphur were estimated by the differential oxidation procedure of EVANS[5]. The combined nitric and perchloric acid digestion method of EVANS *et al.*[6] was used for the determination of total sulphur in the food and in the faecal samples.

The relevant data bearing on the nitrogen and sulphur balance of the animals on the germinated and raw soya bean protein diets are presented in the following tables.

TABLE I

THE NITROGEN BALANCE OF RATS ON THE TWO DIETS

Rat No. and Sex	Total endogenous urinary nitrogen in mgs	Total endogenous faecal nitrogen in mgs	Total intake of nitrogen in mgs	Urinary nitrogen in mgs	Faecal nitrogen in mgs	Digestibility coeff.	Biological value
Germinated protein							
1 M	320	133	2240	1144	525	82.6	55.4
2 M	346	146	2625	1295	578	83.6	56.7
3 M	368	138	2146	1187	426	86.6	55.9
4 M	386	140	2248	1228	468	85.4	56.2
5 M	369	128	2040	1161	412	86.1	54.9
6 M	394	149	2326	1288	535	83.4	53.9
					Average	84.6	55.5
Raw protein							
1 M	320	133	1885	1062	484	82.3	51.6
2 M	346	146	2408	1317	560	82.8	51.3
3 M	368	138	2242	1316	458	85.7	50.7
4 M	386	140	2212	1280	487	84.3	52.1
5 M	369	128	2125	1251	449	84.9	51.1
6 M	394	149	2346	1407	473	86.2	49.9
					Average	84.3	51.3

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TABLE II

UTILISATION OF TOTAL SULPHUR

Rat No. and Sex	Total intake in mgs	Faecal excretion in mgs	Percentage absorption	Urinary excretion in mgs	Percentage retention
Germinated protein					
1 M	139.2	48.6	65.1	76.2	15.9
2 M	163.1	51.8	68.2	94.5	15.1
3 M	133.4	45.1	66.2	75.8	14.2
4 M	139.7	51.6	63.1	76.1	13.6
5 M	126.8	49.8	60.7	64.6	16.2
6 M	144.5	46.5	67.8	86.5	11.7
		Average	65.2		14.5
Raw protein					
1 M	117.1	40.7	65.2	69.6	8.9
2 M	149.6	51.4	65.6	88.6	9.7
3 M	139.3	46.9	66.3	82.9	10.2
4 M	137.5	53.9	60.8	75.6	9.6
5 M	132.0	46.7	64.6	76.2	10.7
6 M	145.7	46.4	68.1	86.5	12.9
		Average	65.1		10.3

TABLE III

URINARY PARTITION OF SULPHUR

Rat No. and Sex	Total intake in mgs	Total Urinary excretion in mgs	Inorganic sulphur in mgs	Ethereal sulphur in mgs	Cystine sulphur in mgs	Methionine sulphur in mgs
Germinated protein						
1 M	139.2	76.2	53.3	8.1	12.2	2.6
2 M	163.1	94.5	69.1	9.8	12.9	2.7
3 M	133.4	75.8	51.0	8.2	15.2	1.4
4 M	139.7	76.1	57.2	7.7	9.9	1.3
5 M	126.8	64.6	45.2	6.1	10.1	3.2
6 M	144.5	86.5	60.5	9.4	15.1	1.5
Raw protein						
1 M	117.1	69.6	49.7	7.2	8.3	4.4
2 M	149.6	88.6	61.9	8.9	11.7	6.1
3 M	139.3	82.9	59.8	8.7	9.2	5.2
4 M	137.5	75.6	54.7	7.5	8.5	4.9
5 M	132.0	76.2	53.1	8.7	8.6	5.8
6 M	145.7	76.5	62.3	9.2	10.1	4.9

The data on the nitrogen balance presented in Table I show that though the digestibility coefficients of the two proteins are the same, the biological value of the protein of the germinated bean is slightly higher than that of the raw ungerminated protein,

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showing that there is a slight increase in the biological value of the protein after germination.

The data on the sulphur balance are very interesting. The percentage of sulphur digested is almost the same for both the raw and the germinated proteins. The percentage of sulphur that is retained after absorption is however slightly higher for the germinated protein as can be seen from the data of the Table II.

The cause for this higher retention of ingested sulphur is explained by the data on the urinary partition of sulphur presented in Table III. It can be seen from the results that while the relative proportion of inorganic and ethereal sulphurs in the urinary excretions is about the same in both the groups, the relative proportion of methionine sulphur excreted is however different in the two cases. The excretion of methionine is definitely larger on the raw protein diet than on the germinated protein diet. The percentage retention of sulphur is therefore higher for the germinated protein than for the raw protein.

Cause for the increased nutritive value

Since it is known that the tryptic inhibitor influences the nutritive value of soya bean protein (HAM *et al.*[10, 11], and WESTFALL AND HAUGE[18]) it was of interest to investigate whether the increase in nutritive value as noted above was due to a decrease in the concentration or activity of the inhibitor during the vital process of germination.

100 g of soya bean was allowed to germinate in a porcelain tray for 72 hours. The bean was then dried in a current of air at a temperature of 40° C. An equivalent quantity of raw soya bean was similarly treated with warm air for the same time. Both the samples were powdered so as to pass through a 60 mesh sieve. The samples were then extracted overnight with four volumes of dilute hydrochloric acid at p_H 4.2. Clear inhibitor extracts were obtained after centrifuging and the reaction was adjusted to p_H 7.6.

2 ml of the inhibitor extracts were then added to 100 ml of cow's milk adjusted to p_H 7.6. A 20% glycerol extract of fresh macerated pancreas served as a source of trypsin. 2 ml of the enzyme extract were added to 100 ml of the substrate.

The mixtures were incubated at 37° C and 5 ml samples withdrawn from the reaction mixtures at the end of 0, 1, 2, 4, 8 and 16 hours were added to an equal volume of 10% trichloracetic acid. The precipitated protein was filtered off and nitrogen in the filtrates was determined. The progress of digestion was thus followed by estimating the nitrogen in the trichloracetic acid filtrates. The results are expressed below in terms of the percentage digestibility of the milk protein.

TABLE IV

PERCENTAGE DIGESTIBILITY BY TRYPSIN

Substrate	1 hour	2 hours	4 hours	8 hours	16 hours
Cow's milk	30.2	46.4	69.2	82.4	89.8
Cow's milk + inhibitor from raw bean	12.9	23.9	32.6	52.4	78.7
Cow's milk + inhibitor from germinated bean	12.9	23.8	32.4	53.6	79.2

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The data in the above table show that the activity of the inhibitor extracts from both the raw and the germinated beans is the same, showing thereby that the inhibitor does not lose its activity during germination. Hence the increase in nutritive value cannot be explained by the concept of the inhibitor. In this case there has been an increase in nutritive value even though the inhibitor is present in the same concentration. The data are therefore to be interpreted to mean that apart from the inhibitor there are still other unrecognized and unexplained factors which affect the utilization of soya bean protein for growth and maintenance of nitrogen balance.

SUMMARY

The utilisation of the nitrogen and sulphur of the proteins of raw and germinated soya beans was determined by balance studies on rats. The digestibility coefficient of the protein was 84.6 and 84.3 and the biological value was 55.3 and 51.3 for the germinated and raw soya beans respectively. The percentage absorption of total sulphur was 65.2 and 65.1 for the raw and germinated beans respectively. The average percentage retention of absorbed sulphur was slightly higher (14.5%) for the germinated bean than for the raw bean (10.3%). Analysis of the urinary sulphur showed that the rats excreted a proportionately larger amount of methionine sulphur on the raw bean diet than when they were fed the germinated beans.

Analysis of the raw and germinated soya beans for their tryptic inhibitor contents showed no difference in the tryptic inhibitor activity.

RÉSUMÉ

Nous avons déterminé l'utilisation de l'azote et du soufre des protéines de fèves de soya crues et germées par des études de bilan chez les rats. Les "coefficients de digestibilité" de la protéine étaient de 84.6 et 84.3 et les valeurs biologiques de 55.3 et de 51.3 respectivements pour les fèves de soya germées ou crues. Les pourcentages d'absorption de soufre total étaient de 65.2 et 65.1 pour les fèves crues ou germées respectivement. Le pourcentage de rétention du soufre absorbé était légèrement plus élevé pour les fèves germées (14.5%) que pour les fèves crues (10.3%). L'analyse du soufre dans les urines nous a montré que les rats excrètent en proportion davantage de soufre sous forme de méthionine lorsqu'ils sont nourris par une diète de fèves crues que lorsqu'ils reçoivent des fèves germées.

L'activité du facteur inhibiteur de trypsine est la même pour les extraits de fèves crues et germées.

ZUSAMMENFASSUNG

Die Verwertung von Stickstoff und Schwefel der Eiweisstoffe roher und gekeimter Soyabohnen wurde an Ratten untersucht. Die Verdaulichkeitskoeffizienten des Proteins waren 84.6 und 84.3 und die biologischen Werte 55.3 und 51.3 für gekeimte und rohe Soyabohnen. Der Prozentsatz des Gesamtschwefels, welcher absorbiert wurde, betrug für rohe und gekeimte Bohnen respektive 65.2 und 65.1. Aber der durchschnittlich von dem absorbierten Schwefel zurückgehaltene Anteil war für gekeimte Bohnen etwas höher (14.5%) als für rohe (10.3%). Eine Schwefelbestimmung im Harn zeigte, dass die mit rohen Bohnen gefütterten Ratten mehr Methioninschwefel abscheiden als die mit gekeimten Bohnen gefütterten.

Die trypsinhemmende Aktivität war in Extrakten aus rohen und gekeimten Soyabohnen dieselbe.

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