J. Nutr. Dietet., (1964), 1, 164

THE EFFECT OF SUPPLEMENTING RAW SOYA BEAN MEAL PROTEINS WITH DL-METHIONINE HYDROXY ANALOGUE (MHA) OR DL-METHIONINE ON PROTEIN EFFICIENCY RATIO AND NET PROTEIN UTILISATION IN ALBINO RATS

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(Received 1 Apr 1964)

The failure of raw soya bean meal proteins to support good growth in experimental animals has been known since the classic studies of Osborne and Mendel[1]. The growth depression resulting from inclusion of raw soya bean in the diet has been attributed to the presence of trypsin and growth inhibitors.[2,3] Melnick *et al.*,[4] suggested that the methionine of raw soya bean was liberated more slowly by the proteolytic enzymes of the intestines than the other essential amino acids so that methionine was ineffectively utilized for protein synthesis. It has been shown by a number of workers[5,6] that the addition of methionine or cystine to raw soya bean meal, improves protein utilization to essentially the same extent as optimal heat processing. Recent investigations carried out in our laboratories[7] and elsewhere[8,9] suggest that methionine hydroxy analogue (MHA) is nearly as effective as an equivalent amount of dl-methionine in supplementing diets containing 10-14% soya proteins, as judged by growth experiments in albino rats and chicks. It was therefore considered of interest to find out whether MHA is as effective as dl-methionine in improving the protein efficiency ratio and net protein utilization of raw soya bean meal proteins.

Experimental

Materials:

Raw soya bean meal: A black variety of soya bean grown in Himachal Pradesh, India, was used. The bean was dehusked and the split legume (*dhal*) was ground in hammer mill to pass througha 50 mesh sieve.

Methionine hydoxy analogue: A sample of feed grade MHA supplied by Monsanto Chemical Co. Ltd., U.S.A. was used.

Methionine: A sample of dl-methionine (E. Merck) of high purity was used.

Skim milk powder: An imported sample of spray dried skim milk powder of good quality was used as control for the PER studies.

Amino acid composition: The essential amino acid composition of the proteins of raw soya meal and skim milk powder as determined according to Krishnamurthy *et al.*,[10] is given in Table I. The raw soya bean meal was supplemented with dl-methionine or MHA at a level of 2.0g/16gN.

Table I. *Essential amino acid composition of the proteins of raw soya bean meal and skim milk powder*

Amino acid (g/16gN)	Raw soya bean meal	Skim milk powder	FAO reference protein pattern[1]
Arginine	7.4	3.7	—
Histidine	2.6	2.7	—
Isoleucine	5.8	6.5	4.2
Leucine	7.6	10.0	4.8
Lysine	6.8	7.9	4.2
Methionine	1.4	2.5	2.2
Cystine	1.8	0.9	—
Total sulphur amino acids	3.2	3.4	4.2
Phenylalanine	4.9	4.9	2.8
Threonine	3.8	4.7	2.8
Tryptophan	1.2	1.4	1.4
Valine	5.0	7.0	4.2

[1] FAO Nutrition Studies No. 16, Food and Agriculture Organization, Rome, 1957

Animal Experiments:

Protein efficiency ratio : The PER of raw soya bean flour, with and without methionine or MHA supplementation was determined at 10% level of protein intake according to the rat growth method of Osborne, Mendel and Ferry [11] Skim milk powder was used as a control food for these studies. Four groups of weanling albino rats (28 days old and weighing about 47g) distributed according to randomised block design ignoring litters were kept individually in raised wire screen bottom cages and fed on different diets (Table II) for a period of 4 weeks. Careful records of the weekly increase in body weight and daily food intake of each animal were maintained. Data regarding the growth of the experimental animals and PER of the proteins are given in Table III.

Table II. *Percentage composition of experimental diets*

Ingredients	Nitrogen free diet	Raw soya meal diet	Skim milk powder diet
Raw soya meal[1]	—	19.7	—
Skim milk powder	—	—	27.1
Salt mixture[2]	2.0	2.0	2.0
Vitaminized starch[3]	1.0	1.0	1.0
Groundnut oil	10.0	10.0	10.0
Corn starch	87.0	67.3	59.9

[1] With or without supplementation with dl-methionine or methionine hydroxy analogue (MHA) at a level of 2.0g/16gN
[2] Hubbel, Mendal and Wakeman mixture
[3] Vitaminized starch of Chapman *et al.*

Composition of liver : After the completion of the PER study, the rats were sacrificed. The livers were removed after bleeding the rats through the abdominal aorta. Histological examination of the livers of rats was carried out according to Tasker *et al.*[12] Total lipids in the liver were estimated according to Hawk and Elvehjem [13]

Net Protiein Utilization : The NPU of the proteins of raw soya bean flour with and without methionine or MHA supplementation was determined at 10% level of protein intake by the method of Miller and Bender.[14] Thirty two male albino rats (28 days old) weighing about 50-54g were allotted to four groups in a randomized block design and fed for a period of 10 days on the experimental diets. The first group of rats received the N-free diet while the three remaining groups received the diets based on either raw soya meal or the same supplemented with methionine or MHA. At the end of the 10 day period, all the animals were sacrificed and analysed for total body protein. NPU values

Table III. *Protein efficiency ratio of raw soya bean meal supplemented with dl-methionine or MHA and the liver lipids of rats receiving the different diets*
(Mean values for 8 male rats per group ; Level of protein 10%)

Source of protein in diet	Initial body weight (g)	Protein intake (g)	Gain in body weight (g)	PER[1]	Liver lipids (%)
Raw soyabean	46.7	14.7	10.6	0.70[3]	4.83[4]
,, +*dl-methionine*[2]	46.5	18.0	35.7	1.98	3.69
,, +MHA[2]	46.6	17.7	35.2	1.94	3.70
Skim milk powder	46.6	24.3	76.0	3.12	3.14
Standard error of the mean				±0.06 (14 df)	±0.17 (20 d f)

[1] Determined for a period of 4 weeks
[2] At a level of 2.0g/16gN
[3] Mean based on 6 observations. Statistical analysis of the above data was carried out, omitting the raw soya bean diet group as one rat died during the course of the experiment and the PER value for another rat was negative.
[4] As one rat died during the course of the experiment statistical analysis was carried out using missing plot technique.

were calculated according to the formula

$$NPU = \frac{\text{Body protein of test group} - \text{Body protein of non-protein group} + \text{protein consumed by non-protein group}}{\text{Protein consumed by test group}}$$

The results are given in Table IV.

Cystine and methionine content of the body: The cystine and methionine contents were estimated in the fat free carcass. Cystine was estimated by the method of Krishnaswamy[15] and methionine was *determined by the McCarthy-Sullivan* method[16] as modified by Bolling.[17] The results are given in Table IV.

Results and Discussion

Amino acid composition (Table I): The proteins of raw soya bean meal contained adequate amounts of all essential amino acids except sulphur amino acids. Supplementation of the soya bean meal with dl-methionine at a level of 2.0g/16gN brought the sulphur amino acid content to about 5 2g/16gN i.e almost to the same level as in egg proteins.

Protein efficiency ratio (Table III): The PER of the proteins of raw soya bean meal was very low (0.70). Out of the 8 rats receiving the raw soya meal diet one rat died and one rat lost weight. Supplementation of raw soya meal with dl-methionine or MHA resulted in a significant increase in the PER of the proteins. There was no significant difference between the PER of raw soya bean proteins supplemented with dl-methionine (1.98) or with MHA (1.94). It is to be observed that the values for the PER of raw soya proteins supplemented with methionine or MHA, at a level of 2.0g/16gN are lower than that (3.12) for milk proteins but comparable to that reported for optimally heat processed soya flour by other workers.[18]

Composition of liver (Table III): The liver sections of rats receiving the raw soya bean meal diet showed generalised fatty infiltration and mild cytoplasmic *vacuolation of protein deficiency type.* The livers of the rats receiving the diets based on skim milk powder or raw soya bean supplemented with dl-methionine or MHA showed a normal picture. The liver lipids (4.83%) of rats receiving the raw soya bean diet was significantly higher than that (3.14—3.70) of rats receiving skim milk powder or methionine or MHA supplemented raw soya bean diets.

Net Protein Utilization (Table IV): The NPU of the proteins of raw soya bean meal was very low (32.9). Supplementation of the raw soya bean meal with dl-methionine or MHA increased the NPU of the proteins to 64 5—65.3. There was however no significant difference between the NPU of raw soya proteins supplemented with dl-methionine (64.5) or with MHA (65.3). The NPU of raw soya proteins supplemented with dl-methionine or MHA are comparable to values reported for processed soya proteins.[14]

Cystine and methionine content of the body: The mean cystine and methionine contents of the whole carcass of rats

Table IV. *Net protein utilization of raw soya meal proteins supplemented with dl-methionine or MHA and the total cystine and methionine contents of the carcass of rats receiving the different experimental diets*
(8 male rats in each group ; duration of experiment 10 days; level of protein in diet. 10%)

Source of protein in diet	Initial body weight (g)	Final body weight (g)	Gain in weight (g)	Mean protein (Nx6.25) intake (g)	Mean carcass protein (Nx6.25) (g)	NPU	Cystine content+ (g)	Methionine content+ (g)
None (N—free diet)	53.5	42.6	—10.9	0.-05	7.58	—	0.134	0.124
Raw soyabean	52.3	53.4	1.1	7 04	9.50	32.9	0.173	0.163
,, +dl-methionine	51.0	69.8	18.8	8.58	12.69	64.5	0.220	0.224
,, +MHA	51.7	70.0	18.3	8.47	12.72	65.3	0.222	0.220
Standard error of the mean						±2.30 (14 d f)	±0.009 (21 d f)	±0.003 (21 d f)

+ Mean values for the whole carcass.

receiving raw soya meal diet were 0.173 and 0.163g respectively; those of rats receiving the methionine or MHA supplemented diets were 0.220—0 222 and 0.220—0.224g respectively. There were no significant differences in the cystine and methionine contents of rats receiving raw soya bean meal supplemented with methionine or MHA thereby showing that MHA had been effectively converted to methionine in the body of the experimental animals.

The results obtained in the present investigation show that dl-methionine hydroxy analogue (MHA) is as effective as dl-methionine in supplementing raw soya proteins and in counteracting the growth depression of the tryptic inhibitors present in the legume.

Summary

1. The protein efficiency ratio (PER) and net protein utilisation (NPU) of raw soya bean meal supplemented with dl-methionine hydroxy analogue (MHA) or dl-methionine at a level of 2.0g/16gN has been determined using albino rats.

2. Supplementation of raw soya bean meal with MHA or dl-methionine brought about a marked improvement in the nutritive value of the proteins The PER and NPU values for the different diets were as follows : raw soya bean meal, 0.70 and 32.9 ; raw soya meal+MHA, 1.94 and 65.3 ; and raw soya meal+dl-methionine 1.98 and 64.5 respectively. The PER and NPU of raw soya meal supplemented with MHA or dl-methionine were comparable to values reported in the literature for optimally heat processed soya bean meal.

3. The methionine hydroxy analogue (MHA) was equally effective as dl-methionine in supplementing raw soya bean proteins and in counteracting the growth depression of the tryptic and growth inhibitors. There was no significant difference in the PER and NPU of raw soya bean meal supplemented with MHA or dl-methionine.

4 The livers of rats receiving raw soya bean meal diet contained significantly larger amounts (4.83) of fat than those receiving the MHA or methionine supplemented diets (3.69—3.70) and showed generalised fatty infiltration and cytoplasmic vacuolation. The livers of rats receiving the supplemented diets were normal.

5. There were no significant differences in the cystine and methionine contents of rats receiving raw soya bean meal supplemented with MHA or methionine thereby showing that MHA could be easily converted to methionine in the body of the experimental rats.

Acknowledgement

The authors are thankful to Mr. A. Paul Jayaraj for the histological examination of the livers and to Mr. A. N. Sankaran for help in the statistical analysis of the results. This study was supported by grants-in-aid from the P.L. 480 funds, U.S. Public Health Service.

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