carried out after separation by solvent extraction. Good recovery was achieved as shown by the average of a number of determinations in Table I. Antimony, could not be determined directly in the benzene layer as the layer showed faint iodine colour even in the reducing atmosphere of ascorbic acid.

Number of developer liquids like water, ethanol + 10% 5 N HCl, butanol + 10 N HCl, pyridine water were tried for chromatographic separation of bismuth and antimony. With the first two developers, both the metals formed a mixed spot. With butanol + 10 N HCl, although the separation was achieved, the spots eluted with KI did not give their characteristic spectra. Antimony and bismuth could be separated with pyridine-water as developer when applied as their nitrates. But when applied as chlorides, a mixed spot was obtained. However, high acid concentration in ethyl methyl ketone containing 10% concentrated HCl reduced the possibility of oxysalt formation and thus resulted in clean separation. The ketone escaped on drying the paper and the spot eluted with KI gave characteristic spectra facilitating their spectrophotometric determination with an error less than 10% in the ppm range.

1. McChesney, E. W., *Ind. Eng. Chem.*, Anal. Ed., 1946, 18, 146.
2. Lure, Yu. Yu. and Ginzburg, L. B., *Zavodskaya Lab.*, 1949, 15, 21.
3. Haddock, L. A., *Analyst*, 1934, 59, 163.
4. Goto, H. and Suzuki, S., *Sci. Repts. Research Insts., Tohoku Univ.*, 1954, 6, 130.
5. Usatenko, Yu. I., Arishkevich, A. M. and Akhmetshiu, A. G., *Zavodok lab.*, 1965, 31 (7), 188.
6. Oosting, M., *Mikrochim. Acta*, 1956, p. 529.
7. West, P. W. and Hamilton, W. C., *Analyt. Chem.*, 1952, 24, 1025.
8. Lederer, M., *Anal. Chim. Acta*, 1951, 5, 185.
9. De Carvalho, R. G., *Paper Read to the International Congress of Analytical Chemistry at Lisbon*, 1956.
10. Pollard, F. H., McOmie, J. F. W. and Elbeith, I. I. M., *J. Chem. Soc.*, 1951, p. 466.
11. "Chromatographic data," *Journal of Chromatography*, 1964, 15, D_1, (Halmekoski, J. and Suomen, F. S., Kemistilehti, 1963, 36 B, 63.

NUTRITIONAL EVALUATION OF SOME INDIAN NONCULTIVATED WILD LEGUMINOUS SEED PROTEIN ISOLATES

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The protein isolates from some uncultivated wild leguminous seeds when supplemented with the limiting amino acids, methionine and tryptophan and fed to animals, no untoward symptoms were noticed and the diets proved capable of promoting growth and maintained positive nitrogen balance in them. Also they did not appear to induce any deleterious physiological after-effects on the animals as evinced by liver protein and several liver enzyme assays.

IN a previous communication[1] a number of wild leguminous seeds were analysed for their chemical composition and their essential amino acid content. Finding their protein and amino acid contents only slightly inferior to casein it was considered pertinent to supplement them with the inadequate amino acids and test their efficiency as growth promoters. However, as their unpalatability, bad odour and toxicity in some cases disallowed feeding of the entire seeds to experimental animals, their soluble proteins were extracted and tested with and without supplementation. The present communication describes the extraction, isolation and purification of proteins from these wild leguminous seeds and their evaluation by animal feeding experiments.

Incidentally, as liver tissue and liver enzymes are the most sensitive to respond to alterations in dietary protein, both qualitatively and quantitatively, liver protein depletion and repletion studies and assays of some important liver enzyme systems that respond significantly during altered conditions of protein feeding, were carried out.

Materials and Methods

Purified protein isolates were prepared as described earlier[2].

Evaluation of Proteins by Animal Experiments.—The biological values of the isolated proteins and their protein efficiency ratios were determined by animal experiments on 12 albino rats per group, 4–5 weeks old and weighing 40–48 g. Both the balance sheet method[3,4] and the rat growth method[5,6] were employed.

Experimental Diets.—A diet practically nitrogen-free but adequate in all other respects was prepared. It contained soluble starch (analytical reagent grade) 80 parts; sucrose, 10 parts; groundnut oil, 6 parts;

salt mixture[7], 2 parts; vitamin mixture, 1 part; and cellulose powder, 1 part. In experimental diets starch was replaced by dietary protein at 10% level.

Vitamin mixture had the following composition per tablet in mg: Inositol, 2·2; Choline chloride, 75; Niacin, 2·0; Riboflavin, 0·4; Pyridoxine-HCl, 0·2; Thiamine-HCl, 0·4; Calcium pantothenate, 1·2; Folic acid, 40 and vitamin B_{12} 600 μg. One tablet of vitamin mixture was employed per 100 g of the diet. Shark liver oil 5 drops per rat twice a week was administered.

All diets were cooked and fed to animals as *detailed earlier*[2].

The liver protein repletion method of Harrison and Long[8] was employed for determining the liver repletion capacity of the wild leguminous seed protein isolates.

Albino rats weighing between 100–160 g were standardized for one week on a diet containing 20% casein. After one week, one group of six animals was sacrificed and total liver protein[9], albumin[10], glycogen[11,12], and activities of some enzyme systems like active phosphorylase[13], succinic dehydrogenase[14], catalase[15], alkaline and acid phosphatases[16,17] and xanthine oxidase[18] were assayed. Subsequently, after fasting all the animals for 48 hours one more group of six animals was sacrificed and all the aforesaid liver assays were repeated. One of the remaining groups of animals was then fed on a 10% casein diet while all the others were maintained on the experimental protein diets at 20% level both in the unsupplemented form as well as supplemented with 0·2% of L-tryptophan and 0·15% of L-methonine. At the end of the experimental period of four days, all the animals (6 in each group) were sacrificed and the liver assayed for total proteins and the various enzyme systems.

Results and Discussion

Most of the experimental unsupplemented protein isolates when fed to the experimental animals failed to promote growth. However, when supplemented with the limiting amino acids methionine and tryptophan, recorded protein efficiency ratios (PER) between 1·0–1·7 and net protein ratios (NPR) between 1·9–2·0 (Table I) as against 2·55 and 3·27 respectively for casein at 10% level. The PER figures are much lower than for casein and also induce less growth (12·6–25·5 g) in comparison with casein (39·8 g) during the same period. The Biological Values (B.V.) and the Digestibility Coefficients (D.C.) range respectively between 43–63 and 72–84 and are not anywhere near for those of casein. This suggests that the wild seed protein isolates although nutritionally much inferior to casein, on supplementing with the limiting amino acids are capable of promoting growth and also maintain nitrogen balance in experimental animals.

Table I

Protein efficiency ratio and net protein ratio of some uncultivated leguminous seed protein isolates

Protein source	Weight change rat in 4 weeks (g)	PER	NPR	B.V.	D.C.
Nitrogen free (12)	−13·5				
Casein (10)	39·8 ± 1·7	2·55	3·27	92·0	96·0
Acacia arabica (12)	− 3·8				
**Acacia arabica* (9)	25·5 ± 4·7	1·72	2·47	52·0	83·0
Acacia catechu (10)	− 6·1				
**Acacia catechu* (11)	17·6 ± 1·7	1·47	2·42	46·0	84·0
Albizzia moluccana (12)	− 7·9				
**Albizzia moluccana* (11)	20·2 ± 3·5	1·45	2·25	43·0	83·0
Albizzia richardiana (10)	21·2 ± 2·5	1·52	2·30	61·6	78·6
Bauhinia macrostachya (8)	− 9·5				
**Bauhinia macrostachya* (8)	14 2 ± 1·6	1·05	2·05	63·0	82·0
Bauhinia malabarica (18)	− 5·3				
**Bauhinia malabarica* (12)	12·6 ± 2·7	1·38	2·85	55·7	75·3
Bauhinia variegata (11)	− 8·1				
**Bauhinia variegata* (9)	16·1 ± 1·8	1·05	1·93	56·6	72·2
Cassia absus (8)	− 4·9				
**Cassia absus* (9)	13·5 ± 2 6	1·00	2·00	47·9	75·8
Leucina glauca (10)	13·3 ± 3·8	1·16	2·10	37·0	78·0
Pithecellobium dulce (11)	− 8·1				
**Pithecellobium dulce* (12)	24·8 ± 2·0	1·54	2·37	62·6	77·5

Figures in brackets denote the number of animals.

* Supplemented with L-Methionine (0·15%) and L-Tryptophan (0·20%). Dietary protein was at 10% level and the rats employed initially weighed between 40–48 g.

Table II shows that rats fed on 20% casein diet for one week had about 21% liver protein. After 48 hours of fasting it declined to 15% which on resumption of the casein diet re-attained almost the original level. On feeding the fasted animals with the unsupplemented diets, the liver protein levels further declined. However, feeding of diets, supplemented with methionine and tryptophan, brought about some improvement in liver protein.

Increase of glycogen in the liver tissue in some cases and depletion thereof in others have been observed during feeding of unsupplemented test diets after fasting period. This could be attributed to the derangement caused in the normal carbohydrate metabolism, probably by the unbalanced amino acid pattern of the diets resulting in the failure to resynthesize the enzymes required for glycogen synthesis and breakdown. This assump-

TABLE II

Repletion studies on liver proteins and glycogen in rats during feeding of protein isolates from some uncultivated leguminous seeds

Diet	Liver wt. (g)	% Liver protein	% Liver albumin	% Liver glycogen (mg %)
20% Casein	4·99±0·61	21·0±3·0	6·6±1·6	344±54
Fasting	3·23±0·53	15·1±1·3	4·8±0·7	84±23
10% Casein	4·75±0·48	19·9±1·3	6·4±1·3	366±83
Acacia arabica	4·31±0·59	16·2±2·3	5·7±2·2	96±29
**Acacia arabica*	5·03±0·76	17·9±3·6	6·0±2·8	223±38
Acacia catechu	4·49±0·67	15·1±2·5	5·6±1·2	100±32
**Acacia catechu*	4·63±0·63	17·0±3·4	6·3±0·9	289±39
Acacia melanoxylon	5·05±0·81	16·5±2·0	4·9±1·7	109±19
**Acacia melanoxylon*	4·38±0·59	17·3±2·2	6·2±2·1	242±23
Albizzia richordiona	6·35±1·10	17·0±4·3	5·3±1·9	205±28
Bauhinia macrostachya	5·86±1·16	13·7±3·1	5·1±1·6	80±28
**Bauhinia macrostachya*	4·49±0·85	14·5±3·4	6·2±2·3	169±41
Bauhinia malabarica	4·86±1·02	13·5±2·6	4·9±1·3	95±21
**Bauhinia malabarica*	3·96±1·01	14·2±2·9	5·8±2·0	179±26
Bauhinia variegata	5·22±0·39	13·8±2·5	3·3±0·4	79±49
**Bauhinia variegata*	4·51±1·48	14·3±0·9	5·4±2·5	189±19
Cassia absus	4·42±0·89	13·6±1·3	4·4±1·1	104±18
**Cassia absus*	5·45±0·98	16·4±1·8	5·2±1·5	153±15
Pithecellobium dulce	4·14±0·43	12·3±1·2	5·0±2·1	93±24
**Pithecellobium dulce*	4·87±0·41	15·6±2·1	5·4±1·2	203±37

* Supplemented with L-Methionine (0·15%) and L-Tryptophan (0·20%).

Studies on liver catalase and alkaline phosphatase (Table III) showed that during starvation the activity significantly increased which on feeding of unsupplemented diets did not improve. However, the supplemented protein diets brought down the activity. Acid phosphatase, succinic dehydrogenase and xanthine oxidase activity during starvation and on unsupplemented incomplete protein diets depicted marked decline which, however, got enhanced on feeding the animals with supplemented diets (Table III).

The enhanced active phosphorylase activity accompanied with liver glycogen depletion could be interpreted in the light of increased metabolic reactions with enhanced rate of glycogen breakdown in the liver tissue. This could also be due to the hormonal stimulation caused by the strain during starvation. However, the overall improvement in all the enzymic activities as well as in glycogen concentration points out and emphasizes the nutritional adequacy of the supplemented test proteins.

tion is partly lent support by an earlier report[19] which points out that accumulation of liver glycogen is characteristic during protein deficiency.

Based on the above investigations, it could be concluded that the wild leguminous seed protein isolates have a fairly balanced amino acid pattern although in most of them methionine and tryptophan happen to be the limiting factors. However, when fortified with the missing essential amino acids, the experimental animals consumed the diets with as much avidity as for any other normal diet and what is more no untoward symptoms whatsoever were noticed in them.

The supplemented protein isolates also proved capable of maintaining positive nitrogen balance and restored normal growth in experimental animals. The results while compared with those obtained for some edible seeds[20] with respect to PER, weight gain, Biological Value and Digestibility Coefficient, etc., indicate the supplemented uncultivated seed protein isolates to be nutritionally at par—if not

TABLE III

Studies on some rat liver enzymes during feeding of protein isolates from some uncultivated leguminous seeds

Diet	Alkaline phosphatase[a]	Acid phosphatase[b]	Active phosphorylase[c]	Catalase[d]	Xanthine oxidase[e]	Succinic dehydrogenase
20% Casein	2·75±1·41	5·31±2·35	3·78±1·91	17·24±1·83	275·0±17·0	2802±86
Fasting	3·5 ±2·41	1·99±1·01	6·03±1·65	24·96±1·25	78·7±11·2	956±28
10% Casein	2·16±0·97	4·28±0·61	4·24±0·85	16·53±2·12	176·9±11·2	2132±71
Acacia arabica	5·40±2·75	2·91±0·23	5·63±1·32	19·92±2·25	89·5±10·9	1505±13
**Acacia arabica*	4·62±2·32	4·87±0·65	3·93±0·69	18·11±1·63	132·6±15·4	2430±86
Acacia catechu	5·65±2·72	2·88±0·16	4·98±0·86	20·76±1·06	73·9±13·6	1480±18
**Acacia catechu*	4·91±1·62	5·49±1·36	3·71±1·13	19·01±1·78	152·4±16·2	2005±13
Acacia melanoxylon	4·32±1·16	2·32±2·13	5·95±1·32	19·98±2·01	115·0±14·6	1932±15
**Acacia melanoxylon*	4·00±1·93	4·45±1·64	4·08±0·93	18·26±1·59	154·0±18·9	2020±21
Albizzia richardiana	4·11±1·26	2·93±1·83	4·51±0·75	16·66±6·20	129·0±17·1	1978±14
Bauhinia macrostachya	6·76±6·02	3·16±0·36	5·23±1·25	19·49±3·37	69·8±15·6	2220±25
**Bauhinia macrostachya*	5·64±3·14	5·30±0·70	4·22±0·78	18·62±1·86	148·6±12·9	2495±32
Bauhinia malabarica	6·85±2·14	4·30±1·18	5·99±1·42	22·13±3·82	65·9±10·6	2380±29
**Bauhinia malabarica*	5·12±1·42	4·60±0·91	4·62±0·89	18·44±3·08	152·3±12·1	2415±53
Bauhinia variegata	5·47±1·72	1·02±0·12	4·50±0·73	19·56±2·19	79·3±14·1	1839±46
**Bauhinia variegata*	4·45±1·35	3·41±1·27	3·81±1·24	16·83±1·96	161·1±12·3	2125±86
Cassia absus	7·98±0·93	3·90±1·13	6·10±0·92	19·49±7·15	109·0± 9·3	1895±17
**Cassia absus*	4·92±0·74	5·20±2·17	4·30±1·00	16·33±4·77	180·0±13·6	2243±21
Pithecellobium dulce	4·96±0·59	1·36±0·11	5·26±0·94	21·95±3·18	98·3±11·1	1070±89
**Pithecellobium dulce*	3 95±1·07	3·50±0·64	3·50±0·56	18·59±2·06	169·0±12·7	1517±53

* Supplemented with L-Methionine (0·15%) and L-Tryptoyhan (0·20%).

[a, b] mg P liberated/g liver protein/hour, [c] μg P liberated/mg liver protein/30 min., [d] Residual O_2 in mg after 10 min. catalase activity at 2° C/g liver protein, [e] μl O_2 consumed/g wet liver/hour, [f] μg formazan formed/mg wet liver.

better—with the edible seeds. Furthermore, they do not appear to induce any deleterious physiological after-effects on the experimental animals, as evinced by liver protein and several liver enzyme assays.

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1. Pant, Radha, Nair, C., Rajagopalan, Singh, K. S. and Koshti, G. S., *Curr. Sci.*, 1974, 43, 235.
2. — and Tulsiani, D. R. P., *Agri. Food Chem.*, 1969, 17, 361.
3. Chick, H., Boas-Fixsen, M. A., Hutchinson, J. D. C. and Jackson, H. M., *Biochem. J.*, 1935, 29, 1712.
4. Martin, C. J. and Robinson, R., *Ibid.*, 1922, 16, 407.
5. Osborne, T. B., Mendel, L. B. and Ferry, E. L., *J. Biol. Chem.*, 1919, 37, 223.
6. Swaminathan, M., *Ind. J. Med. Res.*, 1937, 25, 57.
7. Hubbell, R. B., Mendel, L. B. and Wakeman, A. J., *J. Nutr.*, 1937, 14, 273.
8. Harrison, H. C. and Long, C. H., *J. Biol. Chem.*, 1945, 161, 545.
9. Hawk, P. B., Oser, B. L. and Summerson, W. H., *Practical Physiological Chemistry*, 12 Ed., J. and A., Churchill, London, 1954, p. 550.
10. Weichselbaum, T. E., *Amer. J. Clin. Pathol. Technical Section*, 1946, 10, 40.
11. Good, C. A., Kramer, H. and Somogyi, M., *J. Biol. Chem.*, 1933, 100, 485.
12. Trevelyan, W. E. and Harrison, J. S., *Biochem. J.*, 1952, 50, 298.
13. Cori, C. F., Cori, G. T. and Green, A. A., *J. Biol. Chem.*, 1943, 151, 39.
14. Kun, E. and Abood, L. G., *Science*, 1949, 109, 144.
15. Hawk, P. B., Oser, B. L. and Summerson, W. H., *Practical Physiological Chemistry*, 12th Ed., J. and A., Churchill, London, 1954.
16. Bodansky, M., *J. Biol. Chem.*, 1933, 101, 93.
17. Shinowara, G. Y., Jones, L. M. and Reinhart, H. L., *Ibid.*, 1942, 142, 921.
18. Muramatsu, K. and Ashida, K., *J. Nutr.*, 1962, 76, 143.
19. Durbin, P. A. J., Heard, C. R. C. and Platt, B. S., *Proc. Nutr. Soc.*, 1960, 19, 35.
20. Pant, R. and Kapur, A. S., *Ann. Biochem. and Exptl. Med.*, 1963, 23, 457.