# NUTRITIONAL PROPERTIES OF RAPESEED PROTEIN CONCENTRATE PREPARED FROM COMMERCIAL RAPESEED CAKE

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Rapeseed protein concentrate, free from allyl isothiocyanate, was prepared from locally available rapeseed cake. Protein content and net protein utilization (NPU) of the protein concentrate were found to be 70.0% and 63.6% respectively as against 35% and 53% of the original flour prepared from rapeseed cake, showing marked improvement in the quality and quantity of the rapeseed protein after processing rapeseed flour into the protein concentrate. When mixed with fish protein concentrate or skim milk powder so that the ratio of proteins from either source is equal, the NPU was improved to the level of animal protein employed. Growth rate and protein efficiency ratio of a semi-synthetic diet containing rapeseed protein concentrate were comparable to a casein diet used under the same conditions as control. Reproductive performance of rats fed on rapeseed protein concentrate diet were also found satisfactory.

Rapeseed grows abundantly in Pakistan (31,3,481 tons/year).[1] The rapeseed cake obtained after the extraction of oil is used as a cattle feed. The presence of the toxic glucoside singrin and its hydrolytic product identified by Etlinger and Hodgkins[2] and Kjder *et al.*[3] as allycarbonyl isothiocyanate preclude its use as human food. The toxic effects of this compound are not well defined and most of the evidence is of indirect nature. Manns and Bowlard[4] found that the conception rate in pigs and rats was adversely affected by feeding rapeseed oil meal. The deleterious effect of rapeseed meal on the growth of various species of animals has also been noted.[5] Several investigators[6–8] have reported the successful removal of toxic principles and the preparation of an edible flour for human consumption. However, the flour produced may still suffer from the disadvantage of containing a good deal of indigestible carbohydrates which might swell in the stomach and impair the digestion of children and invalids for whom the high protein food is normally intended. The indigenous cake is produced and stored under unhygienic conditions and contains a lot of dust and dirt. It is doubtful if the flour produced from it will be suitable for human consumption. Keeping in view the toxicity of rapeseed cake and the health hazard involved in the human consumption of the indigenous rapeseed flour, it was considered advisable to produce a protein concentrate from it which would be free from the disadvantages. Since vegetable proteins are generally inferior to animal proteins due to deficiency of one or more essential amino acids,[9] experiments were carried out to improve its protein value. Growth and reproductive performance of rats fed on the protein concentrate as the sole source of protein were also studied to determine its suitability for human feeding.

## Experimental

*Preparation of Rapeseed Protein Concentrate* (RPC).— Rapeseed cake obtained from the local market was ground to pass an 80 mesh sieve, and defatted by means of petroleum ether (b.p. 60–65°C). The residue was almost freed of the solvent by drying under vacuum and finally in an air oven maintained at 60–65°C. The flour thus produced contained 36.5% protein as determined by Kjeldahl method.[10]

The method for the preparation of protein concentrate from rapeseed flour was the same as reported by Ali *et al.*[11] for the preparation of cottonseed protein isolate from indigenous cottonseed cake. The method consisted in extracting the protein with aqueous alkali and precipitating the protein fraction by acidifying the extract. Several experiments were performed by varying the pH of the alkaline and acidic solutions to get the maximum yield of protein (Table 1).

The yield of rapeseed protein concentrate (RPC) in experiment 10 was almost the same as that in experiment 7, but the loss of protein was higher than that in experiment 7. The conditions of experiment 7 were therefore chosen for the preparation of RPC which are described as follows:-

Rapeseed flour, 100 g, was taken in a beaker and 900 ml N/10 NaOH added to it. This quantity of alkali was found to give the required pH as well as sufficient volume for efficient extraction of the proteins. The mixture was stirred occasionally and kept for 4-6 hr at room temperature (25°C). During this period the pH was maintained at 10 by adding a few ml N/10 NaOH. The mixture was centrifuged and the supernatant liquid was decanted off. The residue was transferred to a beaker and stirred with 900 ml water. The pH was adjusted at 10 by adding the required amount of N/10 NaOH. After keeping the mixture for 6 hr as before, the mixture was again centrifuged and the aqueous portion was decanted off. The alkaline extracts obtained from the above processes were combined and acidified with

2N $H_2SO_4$ until pH 4 was reached. The coagulated protein thus obtained was separated by centrifuging and washed repeatedly with water until pH 6.5 was reached. It was treated with alcohol to remove water and pressed against filter paper and dried in an air oven at 50°C. The dried cake was ground to powder to pass on 80 mesh sieve.

*Determination of Allyl Carbinyl Isothiocyanate in RPC.*—Allyl isothiocyanate in the protein concentrate was determined according to the method of Welter[12] and was found absent.

*Determination of Net Protein Utilization (NPU) in Rapeseed Flour and RPC.*—The rapeseed flour and RPC were mixed in a semisynthetic diet containing vitamins and minerals in such a way that protein content of the diet was 10% (11.2% on dry weight basis).

Net protein utilization (NPU) was determined according to the method of Miller and Bender[13] using male albino rats weighing 30–35 g. The composition of the experimental diets and the results of NPU determinations are shown in Table 2.

*Supplementation of RPC with Fish Protein Concentrate and Skim Milk Powder.*—RPC was mixed with fish protein concentrate (fish four) and skim milk powder in such a manner that there was equal contribution of protein from either of the protein sources and that the total protein content in the semisynthetic diets as determined by Kjeldhal method[10] was approximately 10%. The composition of various diets is shown in Table 3.

Skim milk powder was obtained through the courtesy of the Director of Health Services, Lahore. Fish protein concentrate was prepared from sun-dried fish obtained from Manzoor and Co., Karachi, according to the method of Alvi *et al.*[14]

NPU of the various diets was determined as described above using male albino rats weighing 40–45 g. NPU values at 10% protein level were converted to NPU (standardized) (Table 4) using the formula:[15]

$$NPU_{st} = \frac{NPU \times 54}{54 - P} - 8$$

where P is protein cal%.

*Weight Gain, Protein Efficiency Ratio (PER) and Reproductive Performance of Rats.*—RPC and casein (B.D.H.) were mixed with a semisynthetic diet containing minerals and vitamins in such a manner that the protein content was *ca* 10%. (The composition of RPC diet is shown in Table 3. The casein diet served as a control and consisted of casein (B.D.H.) 11.5%, maize starch 38.5%, vegetable fat 15%, glucose 15%, potato starch 10% vitamin mixture[16] 5%; Glaxo salt[16] mixture 5%.)

Sixteen albino rats weighing 30–35 g were divided into 2 groups of 8 in such a manner that each group consisted of 6 females and 2 males and that the average weights of rats of both the groups were identical. One group was fed on RPC diet and the other on casein diet. Food and water were given *ad libitum*. One drop of vitaminised oil containing vitamins A, D, E and K[16] was also given to each rat per week. The animals were kept in wire-meshed cages in an air-conditioned room maintained at 80°F (±2°F). Feeding was continued over a period of 8 weeks and records of weight gain and food intake were maintained. PER was calculated by dividing the weight gain with the protein intake during the experimental period. Growth curves of rats fed on the experimental diets are shown in Fig. 1 and the data regarding weight gain and PER are presented in Table 5.

After 8 weeks the group of rats fed on RPC diet were allowed to feed and grow on this diet. After 4–5 weeks on this diet all the female bore litters. The number of baby rats born to each female along with the average weight of litter is shown in Table 6.

## Discussion

The presence of toxic compound allyl isothiocyanate precludes the use of rapeseed cake flour for human consumption. The rapeseed protein concentrate prepared from indigenous cake shows the absence of this toxic compound. The protein content of the concentrate is 70% which is twice that contained in the original flour (Table 2). The biological value of the protein is also increased—the NPU of the protein concentrate increased from 53.0 to 63.6%. This appears to be due to the elimination of the toxic principle which in some way appears to inhibit the maximum utilization of rapeseed protein in the body.

The data presented in Table 4 show that there is no significant difference between $NPU_{st}$ of fish protein concentrate (98.7%) and RPC supplemented with fish protein concentrate (99.6%), and between $NPU_{st}$ of skim milk powder (84.7%) and RPC supplemented with skim milk powder. Thus blending of RPC with equal concentration of protein from either fish protein concentrate or

TABLE 1.—YIELD OF RAPESEED PROTEIN CONCENTRATE UNDER VARYING CONDITIONS FROM 100 g OF THE CAKE (PROTEIN 36.5%).

| Trial | pH of NaOH soln | pH of acidified curd | Yield of protein concentrate g | % protein in concentrate | Protein in concentrate (A) g | Residue left after extraction with alkali g | % protein in residue | Protein left in residue (B) g | Unrecovered protein 36.5−(A+B) g |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 4 | 10.5 | 75.0 | 7. 8 | 56.9 | 30. 0 | 17. 0 | 11.7 |
| 2 | 8 | 5 | 10.0 | 72.0 | 7. 2 | 57.2 | 30. 0 | 17.16 | 12.1 |
| 3 | 8 | 6 | 6.0 | 67.5 | 4. 0 | 57.5 | 30. 0 | 17. 2 | 13.3 |
| 4 | 9 | 4 | 20.0 | 65.6 | 13.12 | 45.5 | 22. 8 | 10.37 | 13.0 |
| 5 | 9 | 5 | 16.1 | 69.6 | 11.25 | 47.5 | 21. 5 | 10.21 | 15.0 |
| 6 | 9 | 6 | 14.6 | 65.8 | 9. 6 | 46.0 | 22. 8 | 10. 5 | 16.4 |
| 7 | 10 | 4 | 25.5 | 70.0 | 17.13 | 40.0 | 18. 9 | 7. 5 | 11.9 |
| 8 | 10 | 5 | 24.2 | 70.0 | 16.95 | 41.0 | 18. 9 | 7. 7 | 11.9 |
| 9 | 10 | 6 | 21.6 | 70.0 | 15. 0 | 42.5 | 18. 9 | 8. 0 | 13.5 |
| 10 | 11 | 4 | 25.8 | 70.0 | 18. 0 | 38.3 | 11.37 | 4. 4 | 14.1 |
| 11 | 11 | 5 | 24.0 | 70.0 | 16. 8 | 38.9 | 11.37 | 4. 3 | 14.4 |
| 12 | 11 | 6 | 23.0 | 67.5 | 15. 5 | 39.1 | 12. 5 | 4. 8 | 16.2 |

TABLE 2.—PERCENTAGE COMPOSITION AND NET PROTEIN UTILIZATION (NPU) OF THE EXPERIMENTAL DIETS CONTAINING RAPESEED FLOUR AND RAPESEED PROTEIN CONCENTRATE.*

| Diets | Rapeseed flour g | Rapeseed protein concentrate g | Maize starch g | NPU at 10% protein level |
|---|---|---|---|---|
| Rapeseed flour(N% ×6.25=36.5) | 27.4 | — | 22.6 | 53.0 |
| Rapeseed protein concentrate (N% ×6.25=70.0). | — | 14.3 | 35.7 | 63.6 |

*The diet contained the following other ingredients also: hydrogenated fat 15 g; glucose 15 g; potato starch 10 g; vitamin mixture[16] 5 g; mineral mixture[16] 5 g.

TABLE 3.—COMPOSITION OF THE EXPERIMENTAL DIETS.

| Diet | Protein source | Protein contributed g | Rapeseed protein concentrate g | Fish protein concentrate g | Skim milk powder g | Maize starch g |
|---|---|---|---|---|---|---|
| 1 | Rapeseed protein concentrate | 10 | 14.3 | — | — | 35.7 |
| 2 | Fish protein concentrate (N% ×6.25=79) | 10 | — | 12.7 | — | 37.4 |
| 3 | Rapeseed concentrate+protein fish protein concentrate | 5+5 | 7.2 | 6.3 | — | 36.5 |
| 4 | Skim milk powder (N% ×6.25=35) | 10 | — | — | 28.6 | 21.4 |
| 5 | Rapeseed protein concentrate+skim milk powder | 5+5 | 7.2 | — | 14.3 | 28.5 |

*The diets also contained vitamins, minerals etc. as shown in Table 2.

TABLE 4.—NPU VALUE OF EXPERIMENTAL DIETS.

| Diet* | Protein% | k cal/g | Protein cal % | NPU at 10% protein level % | $NPU_{st}$ % | |
|---|---|---|---|---|---|---|
| 1 | 10.6 | 4.29 | 9.9 | 63.0 | 69.1 | |
| 2 | 10.0 | 4.0 | 10.0 | 86.9 | 98.7 | Difference insignificant (2, 3) |
| 3 | 10.00 | 4.29 | 9.3 | 89.1 | 99.6 | |
| 4 | 10.0 | 4.0 | 10.0 | 75.5 | 84.7 | Difference insignificant (4, 5) |
| 5 | 10.7 | 4.28 | 10.0 | 78.4 | 88.3 | |

*For composition of diets see Table 3.

Table 5.—Biological Evaluation of Rapeseed Protein Concentrate and Casein Diets.

Mean values for 8 rats (6 females and 2 males per group)

| Source of protein in diet | Protein on dry wt basis% | Four weeks | | | | | Eight weeks | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dried food g | Protein intake g | Wt gain g | PER | Remark | Food intake g | Protein intake g | Wt gain g | PER | Remark |
| Rapeseed protein concentrate | 11.25 | 203 | 22.8 | 58 | 2.55 | Difference insignificant | 422 | 48.0 | 110 | 2.3 | difference insignificant |
| Casein | 11.20 | 183 | 20.4 | 55 | 2.70 | | 412 | 46.0 | 110 | 2.4 | |

Table 6.—Reproductive Performance of Rats fed on Rapeseed Protein Concentrate Diet.

| Female No. | No. of litter | Average wt of litter g |
|---|---|---|
| 1 | 5 | 5.0 |
| 2 | 6 | 5.0 |
| 3 | 6 | 6.0 |
| 4 | 8 (5 born dead) | 4.0 |
| 5 | 4 | 6.0 |
| 6 | 6 | 5.0 |

skim milk powder improved the NPU of the mixture of the level of animal protein used. These results also confirm the earlier findings of Ali *et al.*[17,18] when improvement in the NPU of certain vegetable proteins was affected by supplementation with fish protein concentrate or skim milk powder.

The growth curves of rats fed on RPC and casein diets show that the growth rates in both the groups of rats are similar (Fig. 1). The gain in weights during the 4-week period for the rats fed on RPC and casein diets were 58 g and 55 g, respectively, while for the 8-week period these were 110 for both. PERs of RPC and casein diets were calculated as 2.55 and 2.7, respectively. The figures for the 8-week period were 2.3 and 2.4, respectively. Statistical analysis of the data revealed that there was no significant difference between the PERs of RPC and casein employed. The above results indicate that RPC can be substituted for casein as a source of protein.

The reproductive performance of the rats fed on RPC at 10% level as the sole source of protein was found to be satisfactory. Out of 6 females, 5 bore normal litter of rats, the average weight of which ranged from 5 to 6 g after eliminating female No. 4 which had 5 still births (such still births are not uncommon in our rat colony for the first pregnancy). The average weight of litters ranged from 5 to 6 g which were comparable to those of rats reared on our stock diet (unpublished results).

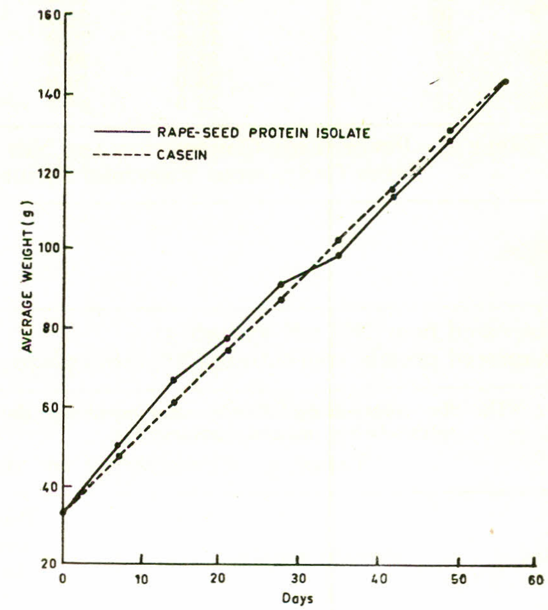


Fig. 1.—Growth curves of rats fed on rapeseed protein isolate and casein diets.

This study shows that rapeseed protein concentrate can be used as a protein source in Pakistani diets as well as in many diet formulae. The residue left after extraction of proteins, amounting to 40% of the original material (Table 1), can still be used as animal feed.

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