REPRODUCTION IN RATS FED ON A GLUCOSINOLATE-FREE RAPESEED PROTEIN CONCENTRATE

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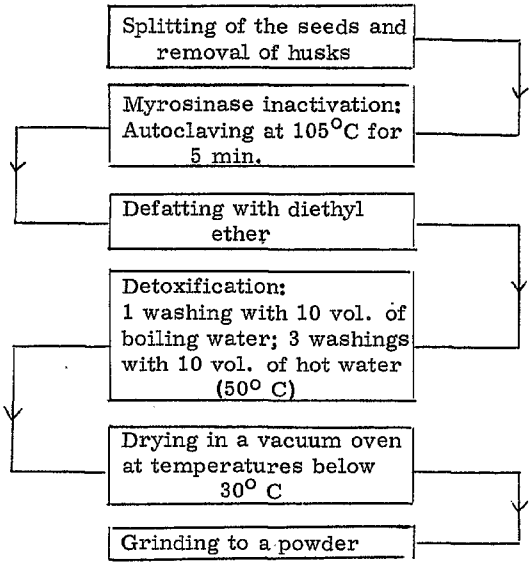
In this paper we are going to report some chemical and biological properties of a protein concentrate prepared from rapeseeds. The seeds used were a Swedish line of winter rape called Sinus. This line has a rather low content of erucic acid. Similar studies which are not included in this presentation have also been carried out with a Swedish line of summer rape called Gulle. Irrespective of the type of rapeseeds used for the preparations, the protein concentrates gave very similar results both with regard to biological protein values and effects produced in pregnant rats.

Table 1 shows a flow-sheet for the preparation of the rapeseed protein concentrate (RPC).

Table 1: Flow-sheet for the preparation of a protein concentrate from rapeseeds.



The seeds were split in a Diamond mill. By means of a vibrating screen and an airstream it was possible to remove most of the husks. In order to avoid enzymatic cleavage of glucosinolates the seeds were autoclaved for 5 min. at 105° C. The husked seeds were then defatted at room temperature by means of extraction with diethyl ether. After careful evapo-

Table 2: Chemical composition of RPC. Average values from 4 different preparations

Constituents		g/100 g of RPC
Moisture		7.8
Ash		6.6
Nitrogen		9.9
Crude protein		61.7
Crude fibre		7.0
Crude fat		0.69
Calcium		0.66
Total phosphorus		1.5
Phytic acid phosphorus		0.68
Iron		0.016
		mg/g of fat-free RPC
VOT[a]	spectrophotometric method	0.0
	GLC-method	0.14
BITC[b]	spectrophotometric method	0.0
	GLC-method	0.03

[a] Vinyloxazolidinethiones
[b] Butenylisothiocyanates

ration of the ether the material was thoroughly washed once with boiling water and three times with hot water at 50° C in order to remove the glucosinolates. The washed material was dried in a vacuum oven and finally ground to a fine powder.

Table 2 shows the chemical composition of the RPC prepared according to this method. It contained approximately 62 % of crude protein and less than 1 % of fat. The crude fibre content was 7 %. About half of the total phosphorus content was in the form of phytate-phosphorus. Calcium and iron amounted to 0.66 % and 0.016 %, respectively.

Using the spectrophotometric method of APPELQVIST and JOSEFSSON (1967) to determine the amounts of vinyloxazolidinethiones (VOT) and butenylisothiocyanates (BITC) released after enzymatic hydrolysis of RPC zero values were obtained. Adopting a more sensitive method using gas-liquid chromatography for the final measurements about 0.14 mg of VOT and 0.03 mg of BITC per g of protein concentrate was found [a]. This corresponded to less than 1 % of the thioglycoside content of the raw material.

The composition of essential amino acids in the Sinus variety rapeseed protein appears from Table 3. It is very well balanced in relation to the pattern of essential amino acids in whole egg. If isoleucine is considered as the first limiting amino acid, the chemical score of the rapeseed protein is 78. The comparatively high content of total lysine (351 mg/gN) as well as "available" lysine (363 mg/g N) should be noted.

The protein efficiency ratio (PER) obtained for the Sinus variety RPC,

[a] Thanks are due to Dr. R. Ohlson, AB Karlshamns Oljefabriker, Sweden, for carrying out the GLC-analyses of VOT and BITC.

Table 3: Composition of essential amino acids in RPC

Amino acid	Egg reference pattern (mg/g of nitrogen)	RPC (mg/g of nitrogen)	Score values for individual amino acids
Isoleucine	415	322	78
Leucine	553	527	95
Lysine	403	351	87
Available lysine	-	363	
Total "aromatic"	627	513	82
Phenylalanine	365	294	80
Tyrosine	262	219	84
Total sulphur-containing	346	284	82
Cystine	149	154	104
Methionine	197	130	66
Threonine	317	313	99
Tryptophan	100	90	90
Valine	454	386	85
Ratio of total essential amino acids to total nitrogen (mg/g)	3215	2786	

Table 4: Protein efficiency ratio (PER) and productive protein value (PPV) obtained with RPC for an experimental period of three weeks. Means and standard deviations of ten male rats per dietary group

Dietary group	Protein content in diet, Nx6.25 (% of fresh weight)	Weight gain g	PER	PPV
Methionine enriched casein [a]	10.0	116.7 ± 11.5	3.83 ± 0.17	65.8 ± 1.8
RPC	10.0	116.9 ± 11.3	3.39 ± 0.13	51.7 ± 1.8

[a] 0.5 g DL-methionine per 100 g of diet

3.4, was slightly lower than that obtained for the reference protein, methionine enriched casein, which gave a PER of 3.8 (Table 4). The productive protein value (PPV) was 52 for RPC and 66 for the casein diet.

Short-term toxicological experiments with detoxified protein concentrates from rapeseeds have recently been reported by us in two papers published in J. Sci. Food Agric. (EKLUND et al., 1971, 1974). In these studies the rapeseed protein seemed practically atoxic to the rat. However, pregnant

rats showed signs of toxic reactions when they were fed on RPC. A short report of this effect was given in a recent issue of Nutr. Rep. Intern. (EKLUND, 1973). Since then more studies on the effects of RPC in pregnant rats have been carried out, and I will now summarize findings which we have made during these experiments.

Table 5 shows the composition of experimental diets. Each diet contained 20 % of crude protein, 5 % oil and standardized levels of fresh vitamin and mineral mixtures. The vitamin E content of the diets generally was 13.4 mg alpha-tocopherol per 100 g. In two dietary groups an extra supply of 200 mg of alpha-tocopherylacetate was given. The crude fibre content was 4 % in each group.

Table 5: Composition of diets used for studies of the reproductive functions in female rats

Constituents, g/100 g	Diets			
	RPC-A	RPC-B	Control-A	Control-B
RPC	32.5	32.5	0	0
Casein	0	0	23.0	23.0
DL-methionine	0.5	0.5	0.5	0.5
Corn oil	5.0	5.0	5.0	5.0
Salt mixture[a]	6.0	6.0	6.0	6.0
Vitamin mixture[a]	2.0	2.0	2.0	2.0
alpha-Tocopherylacetate	0	0.20	0	0.20
Cellulose	2.0	2.0	4.0	4.0
Rice starch	52.0	51.8	59.5	59.3
Analytical data, g/100 g				
Crude protein	20	20	20	20
Crude fat	5	5	5	5
Crude fibre	4	4	4	4

[a] Müller, R. (Z. Tierphysiol. Tierernähr. Futtermittelk. 19, 305, 1964) except for alpha-tocopherol which was included at a level of 13.4 mg/100 g of diet.

The incidence of toxic signs and mortality during gestation, and litter sizes and body weights of the offspring in rats fed on diets containing RPC are given in Table 6. In the group which was fed on the rapeseed protein diet during three weeks before the mating and during the whole pregnancy 5 out of 15 rats died at the term of gestation. Three rats which did not die showed clear signs of toxic reaction. These signs which never appeared prior to the 19th day of gestation were sudden loss of appetite, weight decrease and dark red to black exsudates at the nose and eye-lids. Figure 1 shows the growth curves, obtained with rats fed on the RPC- and control diets, respectively. The appearance of a healthy rat and a diseased rat is illustrated in Fig. 2 and 3.

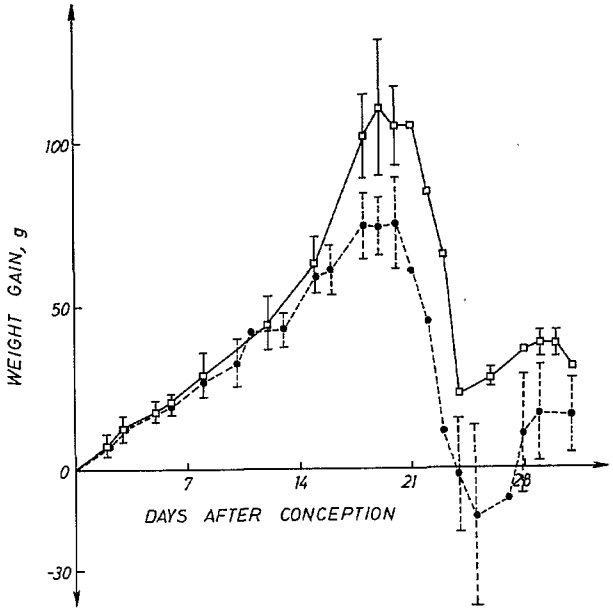
Table 6: Incidence of toxic signs and mortality during gestation, and litter size and body weights of the offspring in rats fed on diets containing RPC or casein as the sole source of protein. Dietary groups are labelled in accordance with table 5. Means ± standard deviations

	Dietary group			
	RPC-A	RPC-B	Control-A	Control-B
No. of rats	15	5	15	5
No. of rats showing toxic signs during gestation	8	0	0	0
No. of rats which died during the experiment	5	0	0	0
Length of gestation, days	22.6±2.2	22.3±0.5	21.8±0.9	21.5±0.6
Maximal weight gain during gestation, g	72±15	75±14	119±23	133±19
No. of litters with live-born pups	8	4	15	5
No. of live pups per litter at parturition	8.6±2.8	8.5±3.9	11.8±3.1	12.3±1.3
No. of live pups per litter 21 days after parturition	3.7±3.9	5.0±3.6	9.3±4.1	11.2±2.3
Body weight of pups 21 days after parturition, g	33.5±9.8	21.9±3.8	45.3±7.6	44.1±6.3

Returning to Table 6 it was found that the length of the pregnancy was not affected but the maximal weight gain of the mother rats was significantly reduced in the RPC-fed rats (72 g) in comparison with the control group given methionine enriched casein (119 g). In the RPC-fed group there also seemed to be a marked reduction in the number of live-born pups per litter, that is 8-9 pups in the RPC-group and about 12 pups in the control group. In the rapeseed group there was also a lower proportion of pups which survived the first three weeks after parturition namely 43 % compared with 79 % in the control group. The pups surviving three weeks had significantly lower body weights than the controls.

5 rats were given an extra supply of vitamin E rising the amount of this constituent from 13.4 mg per 100 g to 213 mg per 100 g of food. The number of rats in this group of course is too small to permit any reliable conclusions to be drawn but it is of interest to note that none of these mother

Figure 1: Growth curves of female rats fed on RPC-(●) or methionine enriched casein-(□) diets during the whole pregnancy. Each group contained 15 rats. In the RPC-group 5 rats died during the experiment. Standard deviations are indicated by the bars.



rats showed signs of toxic reaction. The maximal weight gain, however, was reduced to the same extent as in the other rapeseed group and a similar reduction in the number of live-born pups and survival of pups during the first three weeks was observed. The weight gain of the pups during the first three week period was very low in this vitamin E supplemented group.

Taking into consideration the rather high content of phytic acid in RPC, one might ask if there is not a risk that the experimental animals have a reduced intestinal absorption of some minerals eg. calcium, zinc or iron. Table 7 shows haemoglobin, haematocrit and serum levels of calcium and zinc in mother rats and pups from both a control group and a RPC-fed group. There were only unsignificant differences between these two groups. These results seem to indicate that despite the phytic acid content there is no reduction in the intestinal absorption of at least calcium, zinc and iron in rats fed on diets containing RPC.

Figure 2: Appearance of a control rat during the last days of gestation

Figure 3: Appearance of a diseased RPC-fed rat at the expected time for parturition

Table 7: Haemoglobin and haematocrit values and serum levels of calcium and zinc in mother rats and unweaned pups from RPC- and casein-fed groups, respectively. Dietary groups are labelled in accordance with table 5
Means ± standard deviations

Dietary group	Haemoglobin g/100 ml	Haematocrit %	Calcium mg/100 ml	Zinc mg/100 ml
Mother rats				
RPC-A	13.9±1.8	40±5	12.8±2.5	0.41±0.31
Control-A	12.9±0.7	39±3	14.5±3.6	0.29±0.26
Pups (21 days old)				
RPC-A	10.4±1.4	30±5	9.5±1.3	0.14±0.28
Control-A	10.7±1.5	31±4	10.8±1.7	0.19±0.13

References

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