RAPESEED PROTEIN CONCENTRATES - TOXICOLOGY AND NUTRITION

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For the protein in rapeseed to be acceptable for food, it is imperative that the glucosinolates present be removed. We have devised a method to remove glucosinolates from rapeseed and have evaluated the resulting protein concentrate. Rapeseed glucosinolates give rise to goitrin and isothiocyanate which are the cause of goitre in animals fed rapeseed meals. An indication of the goitrogenic producing characteristics of goitrin and isothiocyanates was achieved by feeding weanling rats a complete casein based diet to which had been added rapeseed extracts containing glucosinolates. By selecting varieties of rapeseed, extracts rich in goitrin (OZT) or isothiocyanate (ITC) yielding glucosinolates, - B. napus varieties for OZT, and B. campestris for ITC, could be prepared and it could be demonstrated that OZT was more toxic than ITC. Thus a diet containing 260 mg OZT per kg and 221 mg ITC per kg caused depressed growth and enlarged thyroid. The same dietary OZT content but with 626 mg ITC per kg, a threefold increase in ITC, did not materially affect rat growth or thyroid enlargement. On the other hand an increase in dietary OZT to 403 mg per kg gave further marked decrease in growth and a large increase in thyroid weight (1) (Table 1).

TABLE 1

GROWTH AND THYROID EFFECTS IN MALE WEANLING RATS FED CASEIN DIETS SUPPLEMENTED WITH RAPESEED EXTRACTS CONTAINING GOITRIN AND ISOTHIOCYANATES. (3 WEEK FEEDING)

Diet	Dietary OZT (mg/kg)	Dietary ITC (mg/kg)	Weight gain (g)	Food consumed (g)	Thyroid weight (mg/100 g)
1	0	0	77.8 ± 3.4	232.6 ± 6.7	5.43 ± 0.23
2	260	221	43.5 ± 3.1	152.1 ± 6.5	8.71 ± 0.61
3	260	626	47.3 ± 4.1	184.9 ± 8.6	9.55 ± 0.71
4	403	972	32.3 ± 4.8	151.6 ± 11.0	13.20 ± 0.91

With this information it was possible to concentrate attention on the OZT content and estimate the level of OZT remaining in rapeseed protein that would cause physiological effects in weanling rats. Young weanling rats were fed a 20 % protein diet based on protein derived from a Rapeseed Protein Concentrate (RPC) from Bronowski rapeseed processed to remove glucosinolates by an aqueous extraction process. Water extracts of rapeseed containing progoitrin were added to the diets to provide OZT levels from 4 to 263 mg per kg diet. Depressed growth and food consumption were observed at 263 mg OZT per kg diet, whereas thyroid enlargement was very evident at 134 mg OZT per kg. One effect of dietary goitrin is to depress food intake which naturally decreases growth. Controlling food intake of a low OZT diet (4.0 mg/kg OZT) to that observed at 263 mg/kg dietary OZT by the pair feeding method results in a similar growth confirming that the growth is controlled by food consumption, whereas thyroid enlargement is due to dietary OZT and the animals response to synthesize thyroxine.

A dietary OZT level of 130 mg/kg was toxic and this could be provided by an RPC with 400 mg OZT/kg (0.4 mg/g or 400 ppm) (1) (Table 2).

TABLE 2

PROGOITRIN ADDITION TO DETOXIFIED RPC DIETS AND THE EFFECT ON GROWTH AND THYROID OF MALE WEANLING RATS. (3 WEEK FEEDING)

Diet	Dietary OZT (mg/kg)	Dietary ITC (mg/kg)	Weight gain (g)	Food consumed (g)	Thyroid weight (mg/100 g)
1	4.0	5.0	105.4[a]	226.1[a]	6.54
2	69.0	74.0	104.4[a]	204.9[a]	8.82
3	134.0	150.9	103.2[a]	202.4[a]	12.14
4	263.0	259.9	89.2[b]	162.4[b]	19.26
1 PR	4.0	5.0	85.4[b]	173.3[b]	5.88
PR	Pair Fed				

Supplied with such information we proceeded to a subacute toxicity evaluation (2). This was conducted according to guidelines published by Health and Welfare Canada for food additives, and those published by the Protein Advisory Group of FAO/WHO/UNICEF for the preclinical testing of novel protein sources. Essentially, test animals were fed a 20 % protein diet in which 20 and 40 % of the protein, usually casein, was replaced by the test material. The test animals, beagle dogs and rats, were fed the diet for 90 days containing an RPC prepared from Echo (B. campestris) rapeseed and containing 0.29 mg/g OZT and 0.90 mg/g ITC. No treatment associated abnormalities were observed in the dogs. Some indication of antithyroid activity was observed in the rats fed the higher level of RPC, manifested as decreased serum thyroxine. The 90 day test with rats was later repeated using a Tower (B. napus) RPC preparation containing 0.02 mg/g OZT (20 ppm) and 0.03 mg/g ITC (30 ppm). No antithyroid or other abnormality was observed in this experiment (2). Thus with rats, an RPC containing 0.29 mg/g OZT used in a subacute toxicity test showed some evidence of antithyroid activity whereas a lower OZT level of 0.02 mg/g RPC was without effect. Thus levels of glucosinolates in RPC without physiological effect in a 90 day feeding test could be achieved with newer varieties of low glucosinolate rapeseed. An OZT content of 0.1 mg/g (100 ppm) seems acceptable in RPC for food use and 20 ppm or less OZT can be readily reached. This compares with contents of 20-30 ppm OZT found in fresh cabbage and up to 150 ppm OZT found in fresh rutabaga (3), which is approximately 200-300 ppm and 1500 ppm OZT respectively on a dry matter basis.

When these experiments were in progress, Eklund (4) reported that RPC produced very damaging effects in pregnant rats, described as loss of appetite, wasting, apathy, bleeding at the eye-lids and nose, leading to reduced litter size and still-born pups. Eklund and Agren further reported on their experiments at the Rapeseed Congress in Giessen (5) in 1974. They showed no deficiency of calcium, zinc or iron that might have been possible in view of the phytate levels in the RPC. They suggested that dietary supplementation with α-tocopherol acetate reduced female rat mortality at the same time indicating that they had insufficient treated animals in their tests to show this.

In 1974, similar pregnant rat tests were performed at the Nutrition Laboratories of Health and Welfare, Canada. Five protein concentrates prepared from rapeseed and one protein concentrate prepared from yellow mustard were used (6). All these preparations gave similar results, - a marked loss of appetite subsequent to day 18 of gestation accompanied by rapid wieght loss and leading to fewer pups born per litter, lower weight of pups at birth and a higher incidence of still-born pups. The more severe symptoms of bleeding from the eyes and nose reported by Eklund were not observed. The phytate levels in the protein concentrate preparations ranged from 5.0 to 7.5 % which alerted us to look for possible trace metal deficiencies. Examination of pooled serum samples from the female rats after parturition for calcium, copper, magnesium, iron and zinc revealed all these to show normal values, except for zinc which was markedly below normal. A zinc deficiency was indicated and the symptoms observed were very similar to those reported by Apgar (7, 8) in reproductive studies in rats on zinc deficient diets.

Evidence to support that a zinc deficiency was the cause of the syndrome observed was obtained by repeating the tests and including a group of animals which received zinc in their drinking water. Table 3 shows the observed results on fetuses, live-born and still-born pups at one, two weeks and at parturition. The decrease in live-born, litter size and increase in still-born pups from females receiving RPC was reversed on zinc supplementation, and animals receiving zinc were comparable to control animals on casein.

TABLE 3

PREGNANT RATS FED RPC. NUMBER OF FETUSES, LIVE-BORN AND STILL-BORN PUPS

	Diet		
	Casein	RPC	RPC + Zn
One week gestation	13.9 ± 1.0	11.4 ± 1.4	11.5 ± 0.8
Two weeks gestation	12.3 ± 1.4	12.8 ± 0.5	13.4 ± 1.2
Live-born	11.1 ± 1.6	7.6 ± 1.5	10.9 ± 1.6
Still-born	0.6 ± 0.2	1.2 ± 0.8	0.3 ± 0.2

Blood serum zinc content of pregnant rats fed RPC were low and about half that in the serum of control animals fed casein. Females receiving zinc and fed RPC showed serum zinc levels comparable to the control animals (Table 4).

These results obtained with a Tower RPC (9) support the fact that the syndrome caused when pregnant rats are fed RPC is caused by a zinc deficiency and that the syndrome disappears when the animals receive zinc in their drinking water. Some component in the RPC must result in the development of zinc deficiency and it is plausible that the phytate present could be responsible. Experiments to test this are in progress. Recently Apgar (10) showed that the zinc deficiency symptoms were overcome by injecting 900 µg zinc at day 18 of pregnancy. Apgar (11) also demonstrated that the symptoms could be generated in pregnant rats with normal zinc status by injecting a chelating agent (EDTA) at day 18 of pregnancy.

The effect of feeding RPC has also been studied with weanling rats (12).

TABLE 4

ZINC CONTENT IN SERUM OF PREGNANT RATS

(μg/g, mean ± S.E.)

Pregnancy state	Diet		
	Casein	RPC	RPC+Zn
Initial	1.39 ± 0.09	0.58 ± 0.06	1.20 ± 0.03
1 week	1.18 ± 0.04	0.64 ± 0.06	1.02 ± 0.04
2 weeks	1.09 ± 0.07	0.59 ± 0.03	1.09 ± 0.13
Post partum	1.10 ± 0.10	0.61 ± 0.06	1.13 ± 0.06

These animals appear to be much less stressed than pregnant rats. The same series of protein concentrates prepared from rapeseeds and yellow mustard were used as protein sources in test diets and a group of animals was included to which supplementary zinc was supplied. Animals receiving RPC showed lower food consumption and weight gain and this effect was removed when zinc supplementation was made, and this gave results comparable to casein control fed animals. In RPC fed animals there were no visible defects but analysis of serum and femus for zinc content showed low zinc values. These values returned to those similar to the control values when zinc was supplemented. Evidently in rapidly growing animals, zinc deficiency does not have the dramatic effect it does in the pregnant rat. Nevertheless RPC fed weanling rats show a marked zinc deficiency.

Recently, Lieden and Hambreus (13) suggested that the pregnant rat syndrome was caused by some factor in RPC other than phytate. They did not indicate if this was a zinc deficiency or whether it responded to zinc supplementation. We have no evidence of any other effect apart from a zinc deficiency in pregnant rats fed RPC.

The protein quality of RPC is very good when assayed by biological tests such as the Protein Efficiency Ratio (PER). PER values equal to casein have been consistently observed and these are substantiated by the essential amino acid composition of RPC preparations.

In the PER assay, no attention has been given to zinc binding factors. When extra zinc was supplied to RPC formulated diets in the PER test, enhanced values were obtained (14) which were superior to those for casein. It appears that protein quality evaluations can be dependent on adequate available zinc in test conditions.

With effective removal of glucosinolate, and by adopting measures to prevent zinc deficiency developing, and provided safety in use can be demonstrated, RPC should become an important food protein ingredient.

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