Rapeseed Protein Concentrate Preparation and Evaluation

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ABSTRACT

Rapeseed is a major oilseed crop in Canada. Many investigators have turned their attention to use of protein left in the meal after oil extraction. However, the meal contains goitrogenic substances which must be removed or inactivated before the meal can be used as a potential protein source for food and feed. A water extraction process has been developed which removes over 90% of the glycosinolates and which results in a protein concentrate having an essential amino acid balance that has been shown by nutritional evaluation to be superior to any other known oilseed protein. However, in highly stressed animals being fed rapeseed protein as the only protein source, a zinc deficiency readily manifests itself, especially with pregnant rats. The zinc deficiency syndrome is readily overcome by supplementing the diets of pregnant rats with zinc in amounts sufficient to complex the phytic acid present in the protein concentrate. This approach appears to be more practical for overcoming the adverse effects of phytates than attempting to remove them by processing treatments. This contribution demonstrates the extensive studies necessary before a protein from a source known to possess toxic and antinutritional factors can be accepted as a food ingredient.

INTRODUCTION

There has been intense activity in recent years to prepare plant proteins in anticipation of their use in foods as alternatives for proteins derived from animal sources, especially meat and milk.

Rapeseed is the most important oilseed crop in Canada. Production statistics indicate that over 68 million bushels are being produced annually in Canada. Development of rapeseed varieties suitable for the short growing season and the climatic conditions has helped to make this possible. Rapeseed is one of the leading potential sources of food protein ingredients based on the production capacity of the crop and the nutritional value of the protein. Interest in rapeseed as a protein source materialized in the sixties when it became generally appreciated that the essential amino acid composition of rapeseed protein compared favorably with that of soybean and the FAO/WHO reference protein (1). However, rapeseed meals have not been used in human food applications due largely to their high levels of toxic compounds and fiber.

Toxic Compounds in Rapeseed

The glucosinolates in the seed are degraded by the enzyme thioglycosidase (myrosinase), also present in the seed, to yield goitrogenic substances (2). These latter substances include: isothiocyanate, nitrile and sulfur or thiocyanate depending on the conditions in the environment. At least seven glucosinolates have been identified in rapeseed (Table I) which range from ca. 10-12 mg/g rapeseed meal. Feeding rapeseed protein meals which contain glucosinolates causes marked effects on growth and thyroid of test animals (Table II).

The nondetoxified rapeseed meal caused striking inhibition of growth and enlarged thyroids. Partial removal of glycosinolate overcomes these effects and results in growth and thyroid effects comparable to those for casein fed animals.

Fiber Content of Rapeseed Meal

The second factor which is a deterrent to use of rapeseed meal as human food is its high fiber content. Rapeseed has a dark, hard seed coat containing a condensed polyphenol-based complex which contributes a substantial amount of fiber to commercial rapeseed meal. Typical crude fibre contents of commercial Argentine and Polish Rapeseed meals are unacceptably high (e.g., 13-16%), and the dark fragments of seed coat in the meal are also objectionable in foods.

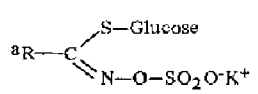
Production of Rapeseed Protein Concentrate

We have been interested in preparing a rapeseed protein concentrate (RPC) containing acceptable levels of fiber and glycosinolates for use in foods. Other investigators, such as Ballester et al. (3), have attempted to remove glucosinolates from commercially processed rapeseed meal. The resulting products are much improved from a feed standpoint, however they are unattractive for food use because of color, flavor and high fiber content. Recognizing that commercial rapeseed meal is unsuitable as substrate for preparing edible protein fractions, we developed the FRI-71

TABLE I

Glucosinolates in Rapeseed

Trivial name	Systematic name	R[a]
Gluconapin	3-Butenyl-glucosinolate	CH_2=CH.CH_2. CH_2-
Glucobrassicanapin	4-Pentenyl-	CH_2=CH.CH_2.CH_2.CH_2-
Glucoraphanin	4-Methylsulphinylbutyl-	CH_3.SO.$(CH_2)_4$-
Glucoalyssin	5-Methylsulphinylpentyl-	CH_3.SO$(CH_2)_5$-
Gluconasturtin	2-Phenylethyl-	C_6H_5.CH_2.CH_2-
Progoitrin	2-Hydroxy-3-butenyl-	CH_2=CH.CH(OH).CH_2-
	2-Hydroxy-4-pentenyl-	CH_2=CH.CH_2.CH(OH).CH_2-



Glucosinolate

TABLE II

Rat Response to Detoxified and Nondetoxified Rapeseed Flour (Span)

Dietary protein (10%)	Weight gain (g per 4 wk)	PER[a]	Thyroid weight (mg/100g b.wt.)
Casein (control)	160.8 ± 5.3[c]	2.50 ± 0.07	6.9 ± 0.56
Nondetoxified rapeseed flour	1.8 ± 0.4[d]	0.1 ± 0.21[d]	29.3 ± 3.18[d]
Detoxified rapeseed flour[b]	152.0 ± 5.7	2.5 ± 0.05	8.07 ± 0.35

[a]Protein Efficiency Ratio.
[b]Contains myrosinase and a 95% reduction of glucosinolates. Experiment was factionally designed where groups of six weanling rats were fed test diets for four weeks. Data shows group means compared with casein control using Student's t-test.
[c]Mean ± S.E.M.
[d]$P < 0.05$. All treatment groups compared to the Casein Control.

TABLE III

Composition and Nutritional Quality of Protein Concentrates of Different Rapeseed Varieties

Dry matter composition %	Echo	Span	Bronowski	Oro	Target	Tower
Fat	4.9	6.1	3.0	7.4	1.0	0.9
Protein	63.4	59.7	69.6	61.8	67.2	66.2
Fibre	6.1	7.7	5.2	6.4	6.0	6.6
Ash	8.8	8.9	8.1	7.3	8.8	10.5
NFE	16.8	17.6	14.1	17.1	17.0	15.8
Glycosinolates[a]						
Isothiocyanates	0.62	0.46	0.02	0.33	0.40	<0.02
Goitrin	0.29	0.16	0.06	0.69	0.63	<0.06
Protein Efficiency Ratio (Casein 2.5)	2.5	2.5	2.8	2.6	—	2.6

[a]Expressed in mg n-butyl isothiocyanate per g. oil-free meal.

process illustrated in Figure 1 which is similar to a process by Ohlson (4). By analogy to soybean technology, rapeseed protein concentrate may be defined as the product prepared from high quality, sound, cleaned, dehulled rapeseed by removal of the oil and water-soluble nonprotein components. In the FRI-71 process, the dehulled seed fraction is subjected to a series of treatments: (a) boiling water treatment to inactivate enzymes in the cotyledons capable of hydrolyzing glucosinolates to toxic compounds; (b) water-leaching to extract water-soluble components, glycosinolates, problem sugars such as raffinose and stachyose, and phenol-like compounds such as sinapine; (c) the extracted cotyledons are recovered, dried and hexane-extracted to yield a protein concentrate with compositions given in Table III.

Rapeseed protein concentrates containing 65-70% protein are readily obtained when the fat is efficiently extracted. Acceptable fiber levels are also achieved. Nutritional evaluation work in our laboratory, using the Protein Efficiency Ratio (PER) method, have consistently shown the rapeseed protein concentrates to be superior to other oilseeds and comparable or superior to casein (Table III). The nutritional excellence is further substantiated by the essential amino acid composistion relative to that proposed by the FAO/WHO reference protein (1). Representative values for the essential amino acids of rapeseed protein concentrates prepared by our process from selected rapeseed varieties are given in Table IV together with the FAO/WHO reference protein.

The more recently developed Target and Tower rapeseed varieties contain improved essential amino acid patterns compared with the older Echo and Bronowski varieties, particularly in leucine, isoleucine, sulfur amino acids and

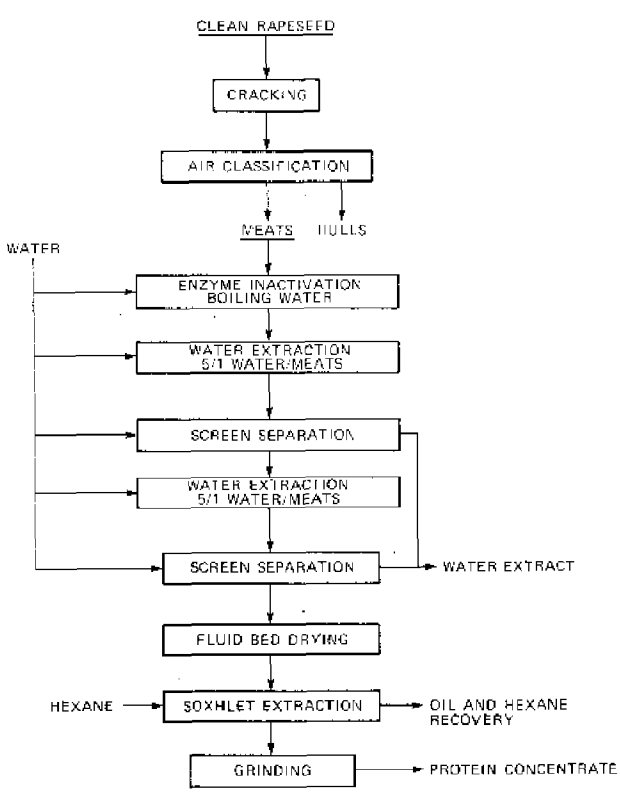


FIG. 1. Flow Diagram of FRI-71 Process.

TABLE IV

Essential Amino Acid Composition of Rapeseed Protein Concentrate (RPC) and FAO/WHO Reference Protein (g/16g N)

Amino acids	Echo[a] *B. campestris*	Bronowski[a] *B. napus*	Target[a] *B. napus*	Tower[a] *B. napus*	FAO/WHO Reference (1973)
Ile	3.8	3.5	4.4	4.1	4.0
Leu	6.7	6.4	7.8	7.3	7.0
Lys	5.9	5.9	5.8	5.8	5.5
Met	2.1 } 3.4	2.0 } 3.7	1.8 } 4.0	2.0 } 4.2	3.5
Cys	1.3 }	1.7 }	2.2 }	2.2 }	—
Phe	3.9 } 6.3	3.9 } 6.2	4.5 } 7.7	4.1 } 7.2	6.0
Tyr	2.4 }	2.3 }	3.2 }	3.1 }	—
Thr	4.1	3.8	4.6	4.8	4.0
Trp	—	—	1.7	—	1.0
Val	5.0	4.7	5.2	5.0	5.0

[a]Rapeseed varieties.

TABLE V

Glucosinolate Content of Rapeseed Flour and Rapeseed Protein Concentrate[a] (mg Aglycone/g Flour)

Rapeseed variety	Aglycone	Rapeseed flour	Rapeseed Protein Concentrate	% Reduction
Span	ITC[b]	7.916	0.460	96.5
	VOT[c]	4.067	0.157	96.2
Target	ITC	4.08	0.400	91.2
	VOT	12.77	0.630	95.1
Oro	ITC	3.740	0.330	91.2
	VOT	3.296	0.688	80.0
Tower	ITC	0.087	<0.030	(>65.5)
	VOT	0.298	<0.060	(>80.0)
Bronowski	ITC	0.480	<0.030	(>93.8)
	VOT	0.660	<0.060	(>91.0)

[a]Detoxification by water-leaching of the glucosinolates.
[b]ITC = isothiocyanates.
[c]VOT = goitrin.

TABLE VI

Mass Distribution (Dry Matter) Bronowski Rapeseed

	Amount
Clean whole seed	100
├→Hulls plus fines	29.3
└→Dehulled seed	70.7
├→Wash solids	16.3
Dehulled, washed	54.4
└→seed (30% protein)	
├→Solvent extract (oil)	29.9
└→Detoxified flour (or protein concentrate) (66% protein)	24.5

threonine. If the sulfur amino acids and aromatic amino acid values are combined, the essential amino acid contents of the Target and Tower concentrates are greater than the FAO/WHO reference (Table IV).

The effectiveness of removing the glucosinolates is shown in Table V. These were achieved with a 5:1 liquid to solids extraction ratio and with two leaching steps. Better than 90% removal of isothiocyanates (ITC) and goitrin (VOT) were achieved. The lower the initial glycosinolate content of the seed, the lower is the residual glucosinolate in the protein concentrate, so that with Tower and Bronowski varieties, the residual glucosinolate levels are less than those measured by routine analytical methods.

The mass balance achieved in the FRI-71 process using Bronowski rapeseed is shown in Table VI. Recent improvements in the dehulling process achieve a 20:80 separation between hulls plus fines and dehulled seed. The yield of protein concentrate from rapeseed is ca. 25%.

Dietary Effects of Glucosinolate

Early in our studies, it was important to have some data on the residual glucosinolate levels that would be acceptable in protein concentrates if zero levels were not readily possible. Since pure glucosinolates were not available for animal tests, we chose water extracts from selected rapeseed processed by the FRI-71 process as a source of glucosinolates. These extracts were concentrated, freeze-dried and chosen on the basis of having progoitrin or glucosinolates other than progoitrin as the dominant glucosinolate present. These glucosinolate sources were added to complete casein-based diets and tested in weanling rats.

Dietary levels of 260 mg goitrin and 221 mg isothiocyanates per kg caused depressed growth and thyroid enlargement (Table VII). Increasing the dietary isothiocyanate to 626 mg while maintaining the goitrin constant at 260 mg did not cause a marked change in growth or thyroid enlargement. Increasing dietary goitrin to 403 mg gave decreased growth and further enlargement of the thyroid. Given this information (5), we added a detoxified protein concentrate, prepared from Bronowski rapeseed, to extracts containing approximately equal levels of progoitrin and other isothiocyanate-yielding glucosinolates. Diets containing 20% protein, all derived from rapeseed protein concentrate, and goitrin levels of 4 to 263 mg per kg of diet were fed for three weeks to male weanling rats (Table VIII). Depressed growth and food consumption were evident at dietary goitrin levels of 263 mg per kg; thyroid enlargement occurred at 134 mg per kg dietary goitrin and possibly even at 69 mg per kg.

Dietary goitrin intake caused depressed food intake. This

TABLE VII

Growth and Thyroid Effects in Male Weanling Rats Fed Casein Diets Supplemented with Rapeseed Extracts Containing Goitrin and Isothiocyanates. (3 Week Feeding)

Diet	Dietary VOT[a] (mg/kg)	Dietary ITC[b] (mg/kg)	Weight gain (g)	Food consumed (g)	Thyroid weight (mg/100g)
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

[a]VOT = goitrin.
[b]ITC = isothiocyanates.

TABLE VIII

Progoitrin Addition to Detoxified Rapeseed Protein Concentrate (RPC) Diets and the Effect on Growth and Thyroid of Male Weanling Rats (3 Weeks Feeding)

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

[a]VOT = goitrin.
[b]ITC = isothiocyanates.
[c]PR = Pair Fed.
[d,e]Means in the same vertical column bearing different subscripts letter are significantly different from each other ($P<0.05$). Unpublished data J.D. Jones.

TABLE IX

Sub-Acute Toxicity Evaluation of Echo Rapeseed Protein Concentrate (RPC) (Thyroxine Concentration in Rat Serum (μg/100 ml))[a]

Treatment group	Sampling day 0	30	60	92
Casein control	6.6 ± 0.3	5.8 ± 0.1[b]	6.4 ± 0.2	5.8 ± 0.3
20% casein replaced with RPC	6.6 ± 0.5	5.9 ± 0.2[b]	6.6 ± 0.3	5.1 ± 0.1
40% Casein replaced with RPC	5.5 ± 0.4	4.6 ± 0.2[c]	5.9 ± 0.3	4.9 ± 0.4

48 rats in each goup, 24 males and 24 females. Data from reference 6.
[a]Data expressed as means ± SE (micro grams per 100 ml).
[b,c]Means in the same vertical column bearing different subscripts letter are significantly different from each other ($p<0.05$).

can be shown by restricting dietary food intake of the diet containing 4 mg goitrin to that of the diet containing 263 mg goitrin using pair fed animals. Thus, a diet containing ca. 130 mg goitrin in a kg caused marked thyroid enlargement but without a marked decrease of food consumed. A goitrin level in a 66% protein concentrate to give 130 mg in a 20% protein diet would need to be ca. 400 mg goitrin per kg of concentrate. This is 0.4 mg goitrin per gram or 400 ppm. Thus, our processing conditions should be such as to yield a product containing less than 0.4 mg goitrin per gram of protein concentrate.

In 1972 we carried out a sub-acute evaluation (6) on rapeseed protein concentrate. This was conducted according to guidelines published by Health and Welfare Canada for food additives (7) and those published by the Protein Advisory Group of FAO/WHO/UNICEF for the preclinical testing of novel protein sources (8). Beagle dogs and rats were fed 90 days a 20% protein diet, usually casein, in which 20 and 40% of the protein was replaced by protein from Echo rapeseed protein concentrate containing 0.29 mg/g goitrin and 0.90 mg/g isothiocyanates. No treatment-associated abnormalities (6) were observed in the beagle dogs, but some indication of antithyroid activity was observed in the rats fed the higher level of rapeseed protein concentrate, manifested as decreased serum thyroxine (Table IX). This test was repeated with a Tower rapeseed protein concentrate containing 0.02 mg/g goitrin and 0.03 mg/g isothiocyanates -- a lower level of residual glycosinolates. No antithyroid effects or any other abnormalities were observed. Goitrin levels, in the range 0.02 to 0.29 mg/g and 0.1 mg/g, seem reasonable to use until other evidence shows otherwise. Levels of 0.02 mg/g goitrin can be readily achieved when using low glucosinolate-containing rapeseed. This compares with up to 0.02 to 0.03 mg/g goitrin found in fresh cabbage and 0.15 mg/g goitrin found in fresh rutabaga (9) (10).

Phytate Effects

When the sub-acute tests were in progress, Eklund (11,12) at Uppsala, Sweden, reported that a rapeseed protein concentrate caused toxic effects in pregnant rats described as a loss of appetite, wasting, apathy, bleeding at

TABLE X

Phytate and Serum Zinc Levels in Pregnant Rats Fed Different Dietary Proteins

Dietary protein	Phytate %	Serum zinc (μg/ml)
Casein	---	1.5
Bronowski	5.3	0.9
Target	6.7	1.0
Tower	7.3	0.7
Yellow mustard	6.4	0.8
Span	7.2	0.6
"Karlshamn"	7.5	0.6

TABLE XI

Serum Zinc Content of Pregnant Rats (μg/g, mean ± S.E.)

Pregnancy status	Diet		
	Casein	RPC[a]	RPC[a] + Zn
Initial	13.9 ± 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

[a]RPC = Rapeseed Protein Concentrate.

TABLE XII

Numbers of Fetuses, Live-Born and Still-Born Pups in Pregnant Rats Fed Rapeseed Protein Concentrate (RPC)[a]

	Diet		
	Casein	RPC	RPC + Zn
One week after pregnancy	13.9 ± 1.0	11.4 ± 1.4	11.5 ± 0.8
Two weeks after pregnancy	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

[a]Experimental details from Reference 16.

the eye-lids and nose, reduced litter size and an increase in numbers of still-born pups. Eklund and Agren (12) also reported on these observations at the Rapeseed Congress in Giessen, Germany in 1974. They suggested the presence of a toxic component other than glucosinolates in rapeseed protein concentrate. In 1974, these tests were repeated at the Nutritional Laboratories of Health and Welfare Canada using protein concentrates prepared from five separate varieties of rapeseed and from yellow mustard (13). All gave similar results -- a marked loss of appetite subsequent to the eighteenth day of pregnancy, accompanied by rapid weight loss and leading to fewer live-born pups, an increase in still-born and lighter weight pups relative to casein control animals. The more severe symptoms of bleeding from the eyes and nose reported by Eklund were not observed. The concentrates contained between 5 and 7.5% of phytate, and this alerted us to look for trace metal deficiencies. Analysis of pooled serum samples from the rats after parturition revealed low zinc values, but normal levels of calcium, copper, magnesium and iron. The serum zinc levels together with the phytate levels of the protein concentrate fed are in Table X. The zinc deficiency observed was similar to that reported by Apgar (14) (15) in reproductive studies in rats on zinc deficient diets. We concluded that the rapeseed and mustard protein concentrates contained components, probably phytate, which caused zinc deficiency.

Additional tests were undertaken in which pregnant rats were fed casein, rapeseed protein concentrate, or rapeseed protein concentrate supplemented with zinc in the drinking water. The same symptoms appeared in the animals receiving rapeseed protein concentrate, but the effect was overcome by zinc supplementation (16). The serum zinc levels of these animals (Table XI) are low for animals fed rapeseed protein concentrate, but return to control levels on zinc supplementation. A marked change in live-born pups and decrease in still-born pups also occurs when the rapeseed protein concentrate diet was supplemented with zinc (Table XII).

The pregnant rat is a heavily stressed animal and shows zinc deficiency very readily towards the end of pregnancy when the fetus grows rapidly and has a large requirement for zinc. Female rats carry no mobilizable zinc in their tissues, and the zinc has to come from the diet. Apgar (17) has recently shown that zinc deficiency in pregnant rats can be overcome by injecting 900 μg zinc at day 18 of pregnancy. Apgar (18) also has demonstrated that the zinc deficiency and its associated symptoms could be brought about in pregnant rats with normal zinc status by injecting a chelating agent (EDTA) at day 18 of pregnancy.

We have, in addition, fed young weanling male rats the same rapeseed protein concentrate preparations used in our first experiments with pregnant rats (19). Zinc was supplemented to one group of animals receiving the protein concentrate, and zinc was determined in the serum and femurs of the animals after three weeks on the diets. The results are shown in Table XIII.

All the animals fed the rapeseed and mustard protein concentrates showed marked decreases in serum and femur zinc content compared to casein-fed controls. Zinc supplementation of the protein concentrate diets elevated serum and femur zinc levels to those of the controls. No visible abnormalities could be seen in the zinc deficient animals, but these animals gained weight at a slower rate than those receiving zinc supplementation or the control animals. In this case, the animals appear not to be subjected to severe stresses as is the case with pregnant animals in a zinc deficient status.

Young weanling rats are used in the Protein Efficiency Ratio Test (PER) to evaluate proteins. No provision is made in the test to ensure that for testing proteins derived from rapeseed no zinc deficiency exists. When extra zinc is supplied in PER tests made with rapeseed protein concentrates, improved values are obtained (20). Additions of 0, 6 and 100 μg of zinc per gram of test diet shows the increases in PER shown in Table XIV. Similar increases are also found in other parameters used to measure the nutritional value as is shown for relative nitrogen utilization (RNU) and relative protein value (RPV). Thus, rapeseed protein concentrate shows very good values for PER and even better values when zinc additions are made to an otherwise zinc deficient status.

Recently, Lieden and Hambreus (21) suggested that the syndrome observed in the pregnant rat when fed rapeseed protein concentrate was caused by some low molecular weight factor in the concentrate. They did not indicate if there existed a zinc deficiency or whether there was any response to zinc supplementation. In our experience the pregnant rat syndrome does not appear if the protein concentrate is supplemented with zinc.

The question remains: is zinc supplementation acceptable as a means for overcoming zinc deficiency in the diet; or possibly phrased differently, is zinc supplementation acceptable as a means for overcoming high levels of phytate in foods to avoid zinc deficiency. Animal tests are continuing to determine this. Phytate is a common component of cereals and oilseeds. It is present in wheat and is known to cause zinc deficiency in man in regions of the world where unleavened bread makes up a large proportion of the diet (22). Phytate appears to selectively bind zinc in foods even when other cations are present.

TABLE XIII

Effect of Zinc Supplementation on Growth and Zinc in Serum and Femurs of Young Rats Fed Rapeseed Protein Concentrate

Dietary protein source	Weight gain (g) Per 100 g feed consumed		Serum zinc (μg/ml)		Femur zinc (μg/ml)	
	-	Zn[a] +	-	Zn[a] +	-	Zn[a] +
Casein	47	47	1.8	1.8	125	129
Protein concentrate:						
Bronowski	41	48	0.6	1.8	36	137
Target	41	47	0.8	1.5	41	117
Tower	38	45	0.5	1.5	34	112
Yellow mustard	41	43	0.7	1.4	43	123
Span	37	44	0.6	1.5	35	123
Karlshamn	41	47	0.8	1.6	38	126

[a]+ 70 μg Zn/ml in drinking water equivalent to ca. 105 μg/g diet; - <5 μg Zn/L in drinking water.

The evaluation tests described illustrate the extensive studies necessary before a protein from a source known to possess undesirable characteristics can be accepted as a food ingredient. If the safety of rapeseed protein concentrate can be demonstrated, there is no reason why this excellent protein should not be used in the food chain.

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TABLE XIV

Zinc Supplementation in Protein Quality Evaluation of Rapeseed Protein Concentrates

	Zinc supplement (μg/g diet)		
	0	6	100
Relative Protein Value	62	81	97
Relative Nitrogen Utilization	85	92	103
Protein Efficiency Ratio	3.07	3.61	4.24

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