NUTRITIONAL QUALITY OF RAPESEED PROTEIN

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The use of rapeseed meal as a protein supplement in animal feeding was extensively reviewed by Bowland et al.(1) In general, the popularity of rapeseed meal for animal feeding has increased despite its disadvantages of being high in crude fibre and thioglucoside content and of having a bitter taste. Primarily because of the above factors Drouliscos and Bowland(2) found rapeseed meal to be inferior in biological value to soybean meal or casein when fed to adult or weanling rats. Although many favourable comments have been made on the balance of amino acids in rapeseed protein(1,2) a study of the protein, free of goitrogenic compounds and produced by a process which is not damaging to the protein, has not been made.

This paper reports on the nutritional quality of a bland, defatted, hull- and thioglucoside-free rapeseed flour which was produced by the Food Research Institute, Canada Department of Agriculture, Ottawa. (3,4,5)

EXPERIMENTAL

The Food Research Institute (FRI) process(3,4,5) was used to produce a flour and meal from Brassica campestris (Echo variety) seed. Briefly, this process consists of wet heat inactivation of the enzyme myrosinase, followed by an aqueous extraction to remove the thioglucosides. The dried meat and seed coat mixture is then solvent-extracted to remove the oil and the defatted material is air-classified into the meats fraction (rapeseed flour) and the seed coat fraction (rapeseed meal).

Whenever commercial rapeseed meals were used in this study, they were obtained by solvent extraction procedures as described by Youngs(1).

Moisture content of the meals was estimated by drying to a constant weight in a vacuum oven set at 80°C. Nitrogen was determined by the micro-Kjeldahl method(6) and a factor of 6.25 used in converting the figures to protein. Fat, ash and crude fibre content of the meals and flour was obtained according to standard methods(7). Amino acid analysis of the protein was conducted according to the method of Moore and Stein(8) and using a Beckman Model 116 amino acid analyzer.

For the estimation of thioglucosides, samples were shaken with a myrosinase solution prepared with phosphate-citrate buffer of pH 7.0. The liberated isothiocyanate and oxazolidinethione were estimated by gas-liquid chromatographic(9) and spectrophotometric(10) methods, respectively.

The main biological test used in evaluation of the protein quality of the materials used in this experiment was the protein efficiency ratio (PER) as described by Chapman et al(11). Casein was used as an internal standard with an assumed PER of 2.50. In most of the studies the diets were fed over a four week period to weanling male Wistar rats, obtained from Woodlyn Farms, Guelph, Ontario. The rats were housed individually in wire mesh cages in an air-conditioned room maintained at 72 ± 2°F. Water and food were supplied ad libitum. Weekly records of weight gain and food consumption were kept.

TABLE I

COMPOSITION OF THE BASAL DIET

	%
Cornstarch	83.7
Corn oil	10.0
Vitamin Mixture(1,3)	2.5
Mineral Mixture(2,3)	3.8

(1)To provide the following per kg of diet: Thiamin HCl, 5 mg; Riboflavin, 10 mg; Calcium pantothenate, 20 mg; Niacinamide, 50 mg; D-biotin, 0.3 mg; Folic acid, 1 mg; Pyridoxine HCl, 10 mg; Vitamin B12, 0.05 mg; Menadione, 0.2 mg; Choline dihydrogen citrate, 5 g; Dry alpha-tocopherol acetate (250 u/g), 0.44 g; Dry vitamin A palmitate (500,000 μ/g), 20 mg; Dry vitamin D_2 (500,000 μ/g), 2 mg; Non-nutritive fibre, 19.44 g.

(2)To provide the following per kg of diet: $CaCO_3$ 7.25 g; $CaHPO_4.H_2O$, 14.30 g; Na_2HPO_4, 6.00 g; KCl, 7.30 g; $MgSO_4$, 2.30 g; Fe $C_6H_5O_7.5H_2O$, 0.59 g; $MnSO_4.H_2O$, 150 mg; $ZnCO_3$, 50 mg; KIO_3, 3 mg.

(3)Obtained from General Biochemicals, Chagrin Falls, Ohio.

The composition of the basal diet is given in Table I. The protein sources were added to the diet to provide a 10% protein content at the expense of the cornstarch. In formulating the diets, the oil content of the meal was considered so that a 10% fat level was uniform in all the diets.

In Experiment 1, samples were obtained of the raw rapeseed (B. Campestris, Echo variety) and the products after enzyme inactivation of the seed, after the aqueous extraction and after the solvent extraction as well as of the final flour and meal. A PER value for these materials was determined, but in a two week test, rather than a four week trial as previously described because of a shortage of the processed material due to the small scale experimental equipment.

The aqueous extract was freeze-dried and the solids incorporated in casein diets at levels identical to their presence in rapeseed. Male weanling rats were fed these diets at levels of 0, 5, 10 and 20% protein for a two-week period. Their growth response was compared with that of animals consuming diets with similar levels of either casein without the aqueous extract solids, or the aqueous extracted defatted meal.

In Experiment 2, the chemical composition and the biological value of three different lots of FRI flour and meal from B. campestris (Echo variety) were determined.

Experiment 3 was designed to compare the protein quality of FRI flour and meal from B. campestris (Echo variety) and B. napus (Bronowski variety) as well as the commercially prepared meals from the two varieties. Flour and meal from the Bronowski variety were produced by the FRI but the enzyme inactivation and aqueous extraction steps were omitted. However, a sample of the Bronowski seed was aqueous extracted and the freeze-dried solids were added to casein diets to determine the toxicity of the extract, as in experiment 1.

Experiment 4 was a comparative study of the PER value of the FRI flour and meal with commercial rapeseed meal, sunflower meal, and soybean meal. Where applicable the data were analyzed statistically using Student's t-test for a comparison of two means or Tukey's procedure for a multiple means comparison(2). The multiple t-test program was designed to run on an IBM 360-65 computer, and critical t values for Tukey's test were obtained by dividing appropriate values from Tables of Studentized Range[13] by $\sqrt{2}$.

TABLE II

PROTEIN CONTENT AND PER DATA OF RAW AND FRI PROCESSED ECHO RAPESEED(1)

Protein Source	Protein(2)	PER(3)
	%	
Rapeseed	24.7	-1.25 ± 0.32(4)
Inactivated rapeseed	23.5	1.14 ± 0.16
Aqueous extracted rapeseed	23.8	2.65 ± 0.09
Rapeseed flour	57.5	2.28 ± 0.04
Rapeseed meal	38.4	1.92 ± 0.04

(1)Data from Tape et al[5]

(2)Air dry basis

(3)Corrected value using casein as an internal standard with a PER of 2.50

(4)Mean ± SEM

RESULTS

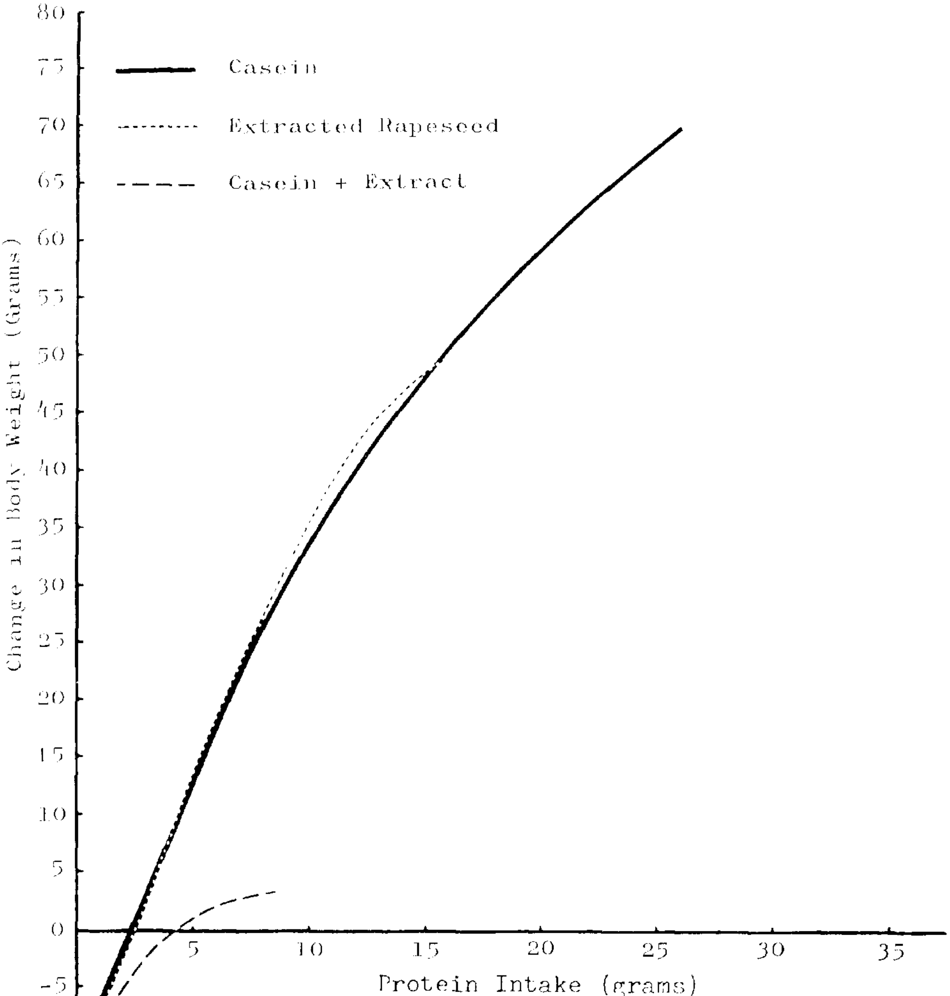
Experiment 1: The protein content and PER values for raw and processed Echo rapeseed are given in Table II. These data show that enzyme inactivation by wet heat, as well as some extraction of thioglucosides which occurs in this process, increased the PER value of the rapeseed protein from -1.25 to 1.14. However, complete aqueous extraction of the thioglucosides was necessary before a PER equal to casein was obtained with the rapeseed preparation. Air classification of the aqueous extracted, defatted meal yielded a high protein (57.5%), good quality flour as well as a 38.4% protein, hull-rich meal.

The effect of adding the freeze-dried thioglucoside-rich aqueous extract to the casein diets is shown in Figure I. This figure also shows the response to the water-extracted meal (23.8% protein) fed at three levels for two weeks. The effect of the addition of the water-soluble extractants to casein is readily apparent as a marked growth depression. In contrast, the extracted meal showed a response comparable to casein.

EXPERIMENT 2

The chemical composition and PER value of three lots of rapeseed flour and meal, prepared over a six-month period are shown in Table III. Although the FRI process was in a very preliminary

FIGURE 1*



EFFECT OF THE AQUEOUS EXTRACT SOLIDS FROM ECHO SEED ON RAT GROWTH

* From Tape et al(5)

TABLE III[1]

PROXIMATE COMPOSITION[2], MUSTARD OIL CONTENT AND PER DATA OF THREE LOTS OF FRI, RSM AND RSF

Product	Protein	Fat	Fibre	Ash	NFE	Isothio-cyanate	Oxazoli-dinethione	PER[3]
	%	%	%	%	%	mg/g	mg/g	
RSF (Jan. 69)	54.7	8.3	8.0	9.5	19.5	nil	0.002	2.67 ± 0.08[4]
RSF (Apr. 69)	54.9	9.1	5.9	7.3	22.3	Trace	0.003	2.54 ± 0.08
RSF (Aug. 69)	52.1	10.9	8.8	9.8	18.4	Trace	0.003	2.56 ± 0.05
$\bar{x}$	53.9	9.4	7.6	8.9	20.1	Trace	0.003	2.59 ± 0.07
RSM (Jan. 69)	34.2	4.3	30.2	4.7	26.6	nil	0.001	2.28 ± 0.04
RSM (Apr. 69)	28.5	4.3	24.6	3.6	39.0	Trace	0.003	2.03 ± 0.03
RSM (Aug. 69)	31.4	5.2	27.1	4.9	31.4	Trace	0.002	2.11 ± 0.04
$\bar{x}$	31.4	4.6	27.3	4.4	32.3	Trace	0.002	2.11 ± 0.04

(1) Data from Tape et al[5]

(2) Dry weight basis

(3) Corrected value using casein as an internal standard with a PER of 2.50 Mean ± SEM

(4) Value significantly different ($P<0.01$) from that of either Apr. 69 or Aug. 69.

(5) Value significantly lower ($P<0.01$) than that of casein or RSF.

experimental stage at the time, the composition of the lots is remarkably similar and the process consistently reduces to a low level the isothiocyanate and oxazolidinethione content of the preparations. The average protein content of the flour and meal was 53.7 and 31.4% respectively. Because of restrictions placed on the process by the equipment available, the fat extraction was not complete so that the meal contained 4.6% fat and the flour contained 9.4% fat. The average PER value of the RSF (2.59) was not different from that of casein but the value obtained for the RSM (2.11) was significantly lower.

TABLE IV

ESSENTIAL(1) AMINO ACIDS(2) OF FRI RAPESEED FLOUR AND MEAL AND OF CASEIN

(% of protein)

	RSF(3)	RSM(3)	Casein	RSM/RSF
Lysine	4.00	4.15	8.10	1.04
Histidine	2.38	1.81	3.32	0.76
Phenylalanine	3.08	2.58	5.19	0.84
Leucine	5.39	4.43	10.24	0.82
Isoleucine	2.52	3.16	4.82	1.24
Threonine	3.01	2.90	4.45	0.97
Methionine	1.51	1.00	2.86	0.67
Valine	3.30	3.14	6.44	0.95
Arginine	4.43	3.56	3.80	0.80
Cystine(2)	0.74	0.70	0.23	0.95
Tyrosine(2)	2.18	1.46	5.79	0.67

(1)Essential for the rat except tryptophan was not determined.

(2)Cystine and tyrosine included because of their replacement value for methionine and phenylalanine, respectively.

(3)Means of three preparations.

The average essential amino acid composition of three lots of rapeseed flour and meal as well as the amino acid composition of casein is shown in Table IV. Examination of the indispensible amino acids in the rapeseed preparations shows that with the exception of arginine, all the amino acids are lower than in casein. However, since recovery of total nitrogen from the column was lower for the rapeseed preparations than for casein, interpretation of the absolute values is difficult. As shown by the relative constancy of the ratio in the last column of Table

IV, RSM and RSF have similar patterns of amino acids. The greatest variations were in methionine and tyrosine which were distinctly lower in the meal, and isoleucine which was higher in the meal.

EXPERIMENT 3

The protein, mustard oil content and the PER values of commercial Bronowski and Echo meal and of the FRI flour and meal from these two rapeseed varieties are shown in Table V. The commercial Echo meal had a high mustard oil content (6.63 and 0.311 mg/g of isothiocyanate and oxazolidinethione, respectively) compared to commercial Bronowski meal. Thus, although they both average 40% protein, the PER values of the commercial Echo meal and Bronowski meal are 1.45 and 2.21, respectively. The FRI flour and meal from the two varieties of rapeseed were similar in protein and goitrogen content, and in protein quality. Both flours gave PER values equivalent to casein.

When a sample of Bronowski seed was put through the FRI aqueous extraction procedure and the freeze-dried solids of the extract added to casein diets, no detrimental effect on rat growth was noted (Figure II).

EXPERIMENT 4

The protein content and PER data for the various oilseed meals as well as the food consumption and weight gain of rats fed these diets are shown in Table VI. The results of the statistical treatment of these data are presented in Table VII.

The weight gain of rats fed casein or FRI Echo flour or meal did not differ with gains of 91.1, 104.0 and 97.9 g respectively, over a four-week period. Soybean meal and sunflower meal diets stimulated greater gains in rats than the commercial rapeseed meals, but lesser gains than the FRI Echo flour or meal, or casein.

Food consumption by rats fed the FRI Echo meal diet was higher at 332.8 g than any of the other groups. Rats consuming the diets containing casein, sunflower meal, soybean meal or the FRI Echo flour ate, on the average, between 278.0 and 291.2 g per rat over the experimental period. During this same period the average food intake of rats fed the commercial rapeseed meals was only 198.7 g.

The PER values of the FRI Echo flour and meal (2.67 and 2.28, respectively) although different from each other, were not

TABLE V

PROTEIN AND MUSTARD OIL CONTENT AND PER VALUES OF MEALS AND FLOURS PREPARED FROM BRONOWSKI AND ECHO VARIETIES OF RAPESEED

Protein Source	Protein(1)	Isothio-cyanates	Oxazoli-dinethione	PER(3)
	%	mg/g	mg/g	
Commercial Bronowski Meal	39.9	trace	0.003	2.21 ± 0.06
FRI Bronowski Flour(2)	55.6	nil	0.003	2.60 ± 0.04
FRI Bronowski Meal(2)	35.6	nil	0.003	1.84 ± 0.05
Commercial Echo Meal	40.5	6.63	0.311	1.45 ± 0.10
FRI Echo Flour (Apr.)	54.9	trace	0.003	2.54 ± 0.08
FRI Echo Meal (Apr.)	28.5	trace	0.003	2.03 ± 0.03

(1)Dry weight basis

(2)FRI steps of wet heat inactivation and aqueous extraction omitted.

(3)Corrected value using casein as an internal standard with a PER of 2.50. Mean ± SEM

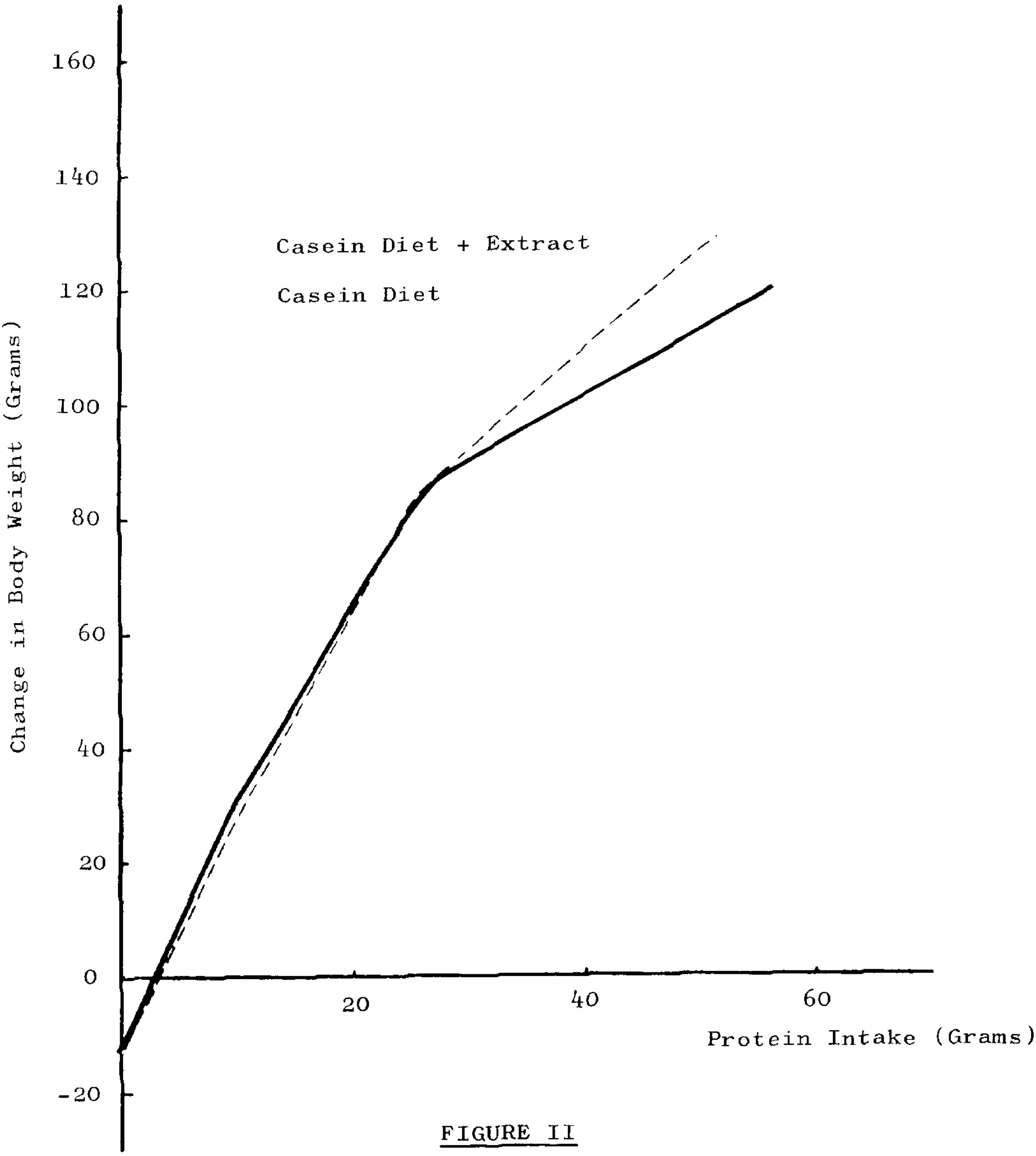


FIGURE II

EFFECT OF THE AQUEOUS EXTRACT SOLIDS OF BRONOWSKI SEED ON RAT GROWTH

TABLE VI

COMPARISON OF NUTRITIONAL INDICES OF RAPESEED MEAL AND OTHER OILSEED MEALS

Group	Protein Source	Protein(1)	Weight Gain	Food Consumption	PER(3)
		%	g/4 wk	g/4 wk	
1	FRI Echo flour (Jan.)	49.0	104.0 ± 5.1(2)	291.2 ± 10.4	2.67 ± 0.08
-	Soyafluff-200C(4)	50.2	-	-	1.78 ± 0.05
2	FRI Echo Meal (Jan.)	32.0	97.9 ± 3.9	332.8 ± 12.0	2.28 ± 0.04
3	Commercial Echo Meal(5)	38.4	55.6 ± 2.5	209.9 ± 3.8	2.05 ± 0.07
4	Commercial Echo Meal(6)	35.6	41.0 ± 4.1	187.5 ± 9.9	1.68 ± 0.11
5	Soybean meal(7)	49.4	76.4 ± 4.1	277.2 ± 9.4	2.12 ± 0.06
6	Sunflower meal(7)	44.6	65.0 ± 2.7	278.0 ± 9.6	1.81 ± 0.05
7	Casein	87.0	91.1 ± 3.7	279.3 ± 7.5	2.50 ± 0.06

(1) Air dry weight basis

(2) Mean ± SEM

(3) Corrected value using casein as an internal standard with a PER of 2.50

(4) Soy flour from Central Soya, Chicago, Illinois. PER obtained during a separate experiment

(5) From Western Canadian Seed Processors, Lethbridge, Alberta

(6) From Saskatchewan Wheat Pool, Saskatoon, Saskatchewan

(7) From Co-op Vegetable Oils Ltd., Altona, Manitoba

TABLE VII

T VALUES FOR MULTIPLE T-TEST COMPARISON OF WEIGHT GAIN, FOOD CONSUMPTION AND PER DATA - EXPERIMENT 4

Group(1)	2	3	4	5	6	7
			Weight Gain			
1	1.13	8.96**	11.67**	5.11**	7.22**	2.39
2		7.83**	10.54**	3.98**	6.09**	1.26
3			2.70	-3.85**	-1.74	-6.57**
4				-6.56**	-4.44**	-9.28**
5					2.11	-2.72
6						-4.83**
			Food Consumption			
1	3.17*	-6.20**	7.91**	1.07	1.01	0.91
2		9.38**	11.08**	4.24**	4.18**	4.08**
3			1.71	-5.13**	-5.20**	-5.29**
4				-6.84**	-6.90**	-7.00**
5					-0.06	-0.16
6						-0.10
			PER			
1	3.92**	6.23**	9.94**	5.52**	8.64**	1.71
2		2.31	6.03**	1.61	4.72**	-2.21
3			3.72**	-0.70	2.41	-4.52**
4				-4.42**	-1.31	-8.24**
5					3.11*	-3.82**
6						-6.93**

(1)Groups 1-7, as given in Table VI

* $P<0.05$ for $t>3.05$

** $P<0.01$ for $t>3.63$

different from casein (2.50). In general the PER values of the commercial rapeseed meals, sunflower meal and soybean meal were lower than casein or the FRI Echo flour.

Soyafluff-200C was fed as a 10% protein diet in another study. However, the corrected PER (1.78) of this soy flour is included in Table VI for purposes of comparison. Possibly the lower PER value of this flour was a result of the moderate heat treatment the flour undergoes during processing.

DISCUSSION

Although there was not enough material available from each stage of processing rapeseed in experiment 1 for a four-week study, the two-week PER values served to point out the improvement in nutritional quality of the rapeseed which was brought about by the FRI process. After the aqueous extraction step the meal is almost free of goitrogenic compounds (Table III). The water soluble goitrogenic compounds are toxic to rats as demonstrated by the depression of growth when the compounds were added to casein diets (Figure 1) and as established by previous studies[1]. Thus the improvement of the nutritional quality of the rapeseed preparation may be largely attributed to the removal of the goitrogens.

In general the FRI RSF contained a protein which gave a PER equivalent to that observed with casein, while the FRI RSM yielded a PER value slightly below casein (Tables III and VI). These results confirmed those of a previous study[2] in which the PER values of commercial rapeseed meal tended to be similar to soybean meal, but growth of the rats was significantly depressed. Drouliscos and Bowland[2] have stated that the poor nutritional quality of commercial rapeseed meals could be attributed to several factors including the goitrogen content, high fibre content and unfavorable amino acid balance. Based on their calculations they suggest that if rapeseed meal supplies the protein in 10% protein diets, the diets are low in lysine, methionine, phenylalanine, valine and isoleucine when compared to the minimum growth requirement of the rat. In comparison to casein, which calculates out to be only low in methionine[2], the FRI RSF and RSM would appear to be low in all the indispensable amino acids except arginine (Table IV). Thus one would expect rats fed the FRI RSF and RSM diet to do relatively poorly. However, as established by the PER data in the present study the protein quality of the FRI RSF and RSM is relatively good. Possibly the amino acid content of the RSM and RSF has been underestimated even though the data on the amino acid composition of casein compare favourably to published data[14]. Clandinin has presented higher values than those presented here for the amino acid composition of commercial rapeseed meals(15).

The food consumption data also support the view that rapeseed protein is of good quality. Rats fed the RSM or RSF diets ate as well and grew as well as the casein-fed rats. If rapeseed protein contained an unfavorable balance of essential amino acids, food intake and hence growth rate would be depressed(16).

Based on the ratio comparing the amino acid composition of RSM to RSF (Table IV) one might predict the performance of rats fed the meal to be less than those fed the flour because of the relatively lower content of certain amino acids in the meal. This viewpoint is supported by the lower PER value of RSM compared to that of RSF. However, food intake of the RSM fed rats was higher than those fed the RSF while both groups grew at the same rate. Thus the relative quality of the RSM diets is obscured to some extent by increased appetite of the RSM fed rats. This increased appetite could probably be attributed to the high (10%) crude fibre content of the FRI RSM diets, which forced the rats to eat more to meet their requirements for calories.

The Bronowski flour and meal were produced from seed grown in Saskatchewan during the 1968 season. These protein sources proved to be of good nutritional quality (Table V) and compared favorably to the FRI Echo flour and meal.

GENERAL CONCLUSION

Rapeseed protein, as it exists in thioglucoside-free and solvent extracted, high-protein flour or hull-rich meal is of good nutritional quality. Although more detailed studies must be conducted to thoroughly assess the nutritional value of rapeseed protein, the results of this study suggest that rapeseed protein has a high potential as a protein source in human food formulations. In such an application the flour, because of its low fibre content, has definite advantages over the meal.

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1) QUESTION: I would like to ask Dr. Aref if he could comment briefly on the acceptability of some of those products they prepared and the comparison between rapeseed and soybean flour?

ANSWER: (Dr. M. Aref)

Roughly there is no difference, between the soya and the rapeseed products.

2) QUESTION: I would like to ask Dr. Anderson, there was a figure in your table about protein efficiency ratios for different rapeseed products after different treatments. You stated for one extracted rapeseed and was this without defatting or after defatting that you obtained a protein efficiency ratio of 2.65?

ANSWER: (Dr. G.H. Anderson)

That was before defatting.

3) QUESTION: I was wondering with regard to the method you are using to get the precision on your oxazolidinethione measurement down to .001? I would like to know if this is a new method or whether you really trust that figure?

ANSWER: (Dr. M. Aref)

These were the figures that were run by Dr. Eapen and by Dr. Downey when he was here. It was the method used by Dr. Downey previously.