NUTRITIONAL EVALUATION OF RAPESEED OIL AND PROTEIN FOR FOOD USE

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INTRODUCTION

Four years ago, the plenary lecture on the nutritional aspects of rapeseed oils dealt with the structure of the oleic acid family of which erucic acid is the C_{22} member (VLES, 1974). Then, the avialable rapeseed oils contained a higher concentration of erucic acid than is encountered in many countries today. The research published in the interval between the conferences has focussed on explanations for the cardiac lipidosis associated with a large intake of erucic acid and on the occurrence and cause of long-term lesions. Consumption of low-erucic rapeseed oil has posed problems in the male rat, as has that of rapeseed protein concentrate in the pregnant rat. Fortunately, some progress has been achieved.

DIGESTIBILITY

The poor digestibility of traditional rapeseed oil was related to its content of erucic acid. In the intestine, there was complete hydrolysis of both high and low erucic rapeseed oils, but free erucic acid was more poorly absorbed than were shorter fatty acids (SERGIEL & ROCQUELIN, 1975).

BLOOD PROPERTIES

In rats fed high or low erucic rapeseed oil some changes were observed, as shown in Table 1.

Table 1: Properties of blood from rats fed rapeseed oil

Property	High erucic oil	Low erucic oil	References
Triglyceride 22:1		-	Rocquelin et al., 1975
Coagulation time	-		Jacquot et al., 1975
Thrombogenicity			Renaud & McGregor, 1976

The coagulation time and thrombogenicity involved comparisons between rapeseed oil and butter. No differences were observed in the clotting time of cynomolgus monkeys fed low rapeseed oil or soybean oil (KRAMER et al., 1978a).

In an attempt to assess the exposure of man to rapeseed oil, seventy Italian railway workers were monitored for serum erucic acid after 12-hour fast (GATTI & MICHALEK, 1975). Under such conditions, low levels of 0.3 to 3.8% erucic acid were detected in the blood of forty subjects.

EFFECTS ON ADRENAL GLANDS

The accumulation of cholesterol erucate in the adrenal cortex was shown to be assiciated with the reduced ability of cholesterol ester hydrolase to act upon the long-chain ester (BECKETT & BOYD, 1975). Compared to cholesterol oleate, the rate of hydrolysis of cholesterol erucate (Fig. 1) was only 25 to 30%.

The debate on the effects of high levels of dietary erucic oil in rats kept in the cold has continued with one group finding increased mortality and another group finding no effect (BEARE-ROGERS and NERA, 1974; HULAN et al., 1976b). During exposure to $4^{o}C$, for two weeks, the level of adrenal corti-

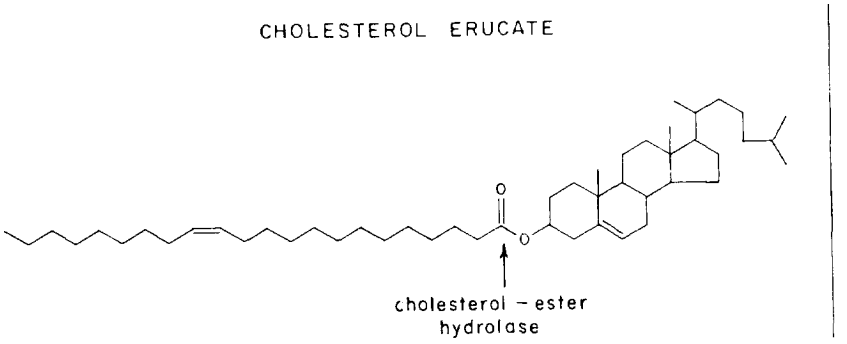

Fig. 1. Hydrolysis of cholesterol erucate.

costerone decreased in rats fed rapeseed oil while not changing in those fed sunflower oil. (BUDZYNSKA-TOPOLOWSKI et al., 1975). In the same investigation, in response to exercise it was observed that the level of adrenal cortisone increased in rats fed sunflower oil but not in those fed rapeseed oil.

CARDIAC LIPIDOSIS

The fatty deposits in the myocardium involved the accumulation of triglycerides containing erucic acid. Further work has added to the literature on lipidosis produced in experimental rats fed high levels of erucic acid (ENGFEDLT & BRUNIUS, 1975a; ASTORG & LEVILLAIN, 1977; BRANCA et al., 1977; ONG et al., 1977; ROCQUELIN et al., 1977). Compared to shorter chain fatty acids, erucic acid underwent slower activation, transfer as its carnitine derivative through the inner mitochondrial membrane, and β-oxidation (SWARTTOUW, 1974; CHENG & PANDE, 1975; BLOND et al., 1975; CHRISTOPHERSON & CHRISTIANSEN, 1975; KIENLE et al., 1976; CHRISTIANSEN et al., 1977; SEHER, 1977). The activity of acyl-CoA dehydrogenase at the first step in β-oxidation was reduced by each additional two carbons on the substrate (KORSRUD et al., 1977). The principal metabolic events are shown in Fig. 2.

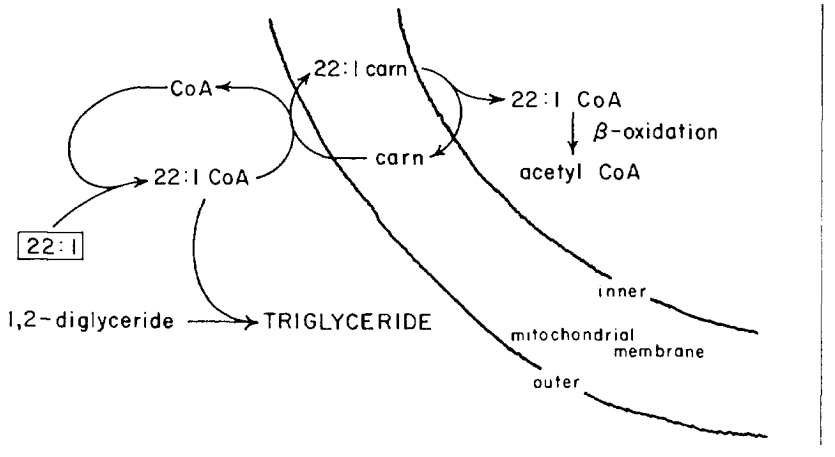

Fig. 2. Metabolic events involved in lipidosis.
(Carn = carnitine).

The unoxidized erucic acid was then largely stored in triglycerides. An increased potential developed for their synthesis from glycerol-3-phosphate (HUNG & HOLUB, 1977a). The activity of acyl-CoA:1,2-diglyceride acyltrans-

ferace increased during the stage of lipidosis and of acyl-CoA hydrolase (lipase) at a later time (HUNG & HOLUB, 1977b).

A deficiency of magnesium provoked cardiac lipidosis, but supplemental magnesium did not prevent it in rats fed high erucic rapeseed oil (POINTILLART & FRANCOIS, 1975; POINTILLART & MESLIN, 1975).

MITOCHONDRIAL FUNCTION

Whether or not mitochondrial function is impaired in rats given a high level of erucic acid has remained a matter of controversy. Washed mitochondria are obviously studied in a different environment from that which exists in the animal. In the presence of heparin, oxidation by cardiac mitochondria from rats fed rapeseed oil was unaffected, but otherwise a reduced rate of oxygen consumption prevailed with substrates of the tricarboxylic acid cycle (DOW-WALSH et al., 1975). Many investigators detected no change in the ability of mitocondria isolated from rats receiving erucic acid to oxidize fatty acid substrates or metabolic intermediates (KRAMER et al., 1973; CHENG & PANDE, 1975; BEARE-ROGERS & GORDON, 1976; KIENLE et al., 1976). On the other hand, mitocondria isolated from rats fed corn oil showed a higher rate of oxygen consumption than those rats fed rapeseed oil (HSU & KUMMEROW, 1977).

Larger and more numerous cardiac mitochondria were also associated with traditional rapeseed oil than with peanut oil (CLOUET et al, 1976). A reduced oxidative capability was accordingly postulated.

MEMBRANE COMPOSITION

An alteration in the composition of cardiac phospholipids in rats supplied with erucic acid has been confirmed. Incorporation of that long chain fatty acid into mitochondrial phosphatidyl choline and phosphatidyl ethanolamine was 5% of the fatty acids while the corresponding concentration in cardiolipin reached 12% (BLOMSTRAND & SVENSSON, 1974). Fatty acids in the mitochondrial membrane fraction containing the activities of the electron transport chain and cytochromes reflected the dietary fatty acids (CLANDININ, 1976).

In vivo and in vitro experiments sometimes yielded different results. Erucic acid accumulated in the free fatty acid fraction of the perfused heart and of tissue slices (VASDEV & KAKO, 1976, 1977). Newborn rat heart cells in culture became appreciably modified in membrane constituents when subjected to erucic acid (PINSON & PADIEU, 1975). In such heart cells 9% erucic acid was incorporated into the fatty acids of phosphatidyl choline; in the corresponding fraction from liver cells erucic acid reached an astonishing 18% (ROGERS, 1977a & b).

In a long term study with high erucic rapeseed oil, the intact rat heart incorporated only low levels of erucic acid into the major phospholipids, but the heart did increase its concentration of sphingomyelin and the content of erucic acid in that fraction (BEARE-ROGERS, 1975; DEWAILLY et al., 1977). The effect on sphingomyelin of the level of erucic acid in rapeseed oil is shown in Table 2. The consequences of such changes in membrane components are still largely a matter of speculation.

Table 2. Cardiac lipids of rats fed for 16 weeks

Dietary Fat mg/ht	Total Fatty Acids mg/ht	22:1 mg/ht	Sphingomyelin P µg/ht
Lard: corn oil	40.8	0	26.3
RSO-3*	53.6	0.3	70.1
RSO-38*	46.5	2.6	145.8

*Percent erucic acid in rapeseed oil

CARDIAC NECROSIS AND FIBROSIS IN THE RAT

That erucic acid per se causes not only diffuse fat accumulation in the heart but also focal necrosis and fibrosis was previously discussed (BEARE-ROGERS, 1975). Even ten years ago, however, Rocquelin and Cluzan (1968), reported lesions in male rats fed low erucic rapeseed oil and proposed that beside erucic acid some other factor contributed to the condition. They pointed to the low level of saturated fatty acids, the high level of linolenic acid and the possible existence of a non-triglyceride component with cardiotoxic properties. The residual erucic acid present in the oil was also regarded as the causative agent (VLES et al., 1976; VLES et al., 1977; ZEMLANSKI, 1977). Ilesmann et al. (1976) partially attributed the lesions observed after feeding Lesira oil (4.2% 22:1) and Erglu (5.2% 22:1) to the content of the long-chain fatty acid.

Further evidence was provided that in the rat the lesions were a problem only in males (VOGTMAN et al., 1975; HULAN et al., 1977c). At fourteen weeks, however, the intake of low erucic rapeseed oil had not influenced the rat electrocardiogram (HUNSAKER et al., 1977).

A summary of results from Canadian studies in which male Sprague Dawley and Wistar rats fed diets containing Span, Oro, Zephyr or Tower oils for at least 16 weeks is shown in Fig. 3-6. The control oils varied and, in individual comparisons involving relatively few animals, were sometimes not significantly different in their effect from rapeseed oil. The general picture, however, is that of a higher incidence of lesions in rats fed the low erucic rapeseed oils than in those receiving the other oils.

The Oro oil has been of particular interest because when diluted 1:1 with other dietary fat it had negligible effect on cardiac tissue (BEARE-ROGERS et al., 1974) and even when it was the only fat in the diet, rat hearts after serial sectioning did not exhibit any lesions (ENGFELDT & BRUNIUS, 1975b; ENGFELDT & GUSTAFSSON, 1975).

The old notion of a balanced fatty acid pattern in the diet has continued to be attractive and illusive. Interest in the fatty acids consumed was reinforced by the observation of lesions in rats fed triglyceride preparations from low erucic rapeseed oil (KRAMER et al., 1975a & b), or by the improvement in the oil after partial hydrogenation (BEARE-ROGERS et al., 1974; CHARLTON et al., 1975; BEARE-ROGERS & NERA. 1977: SLINGER 1977)

The question posed was whether in liquid rapeseed oil, the levels of saturates and linoleic acid relative to linolenic acid were too low. The growing rat appears to have a wide tolerance for fatty acid patterns, as demonstrated by its acceptance of a preponderance of oleic acid in olive oil, or of linoleic acid in sunflower oil or of linolenic acid in linseed oil. A critical balance of fatty acids would have to be envisioned to account for lesions being produced by 5% but not 3% erucic acid added to olive oil (BEARE-ROGERS, 1975; HULAN et al., 1977b). A high intake of linolenic acid was also proposed as a cardiopathogenic agent (McCUTCHEON et al., 1976). Linseed oil containing 61%

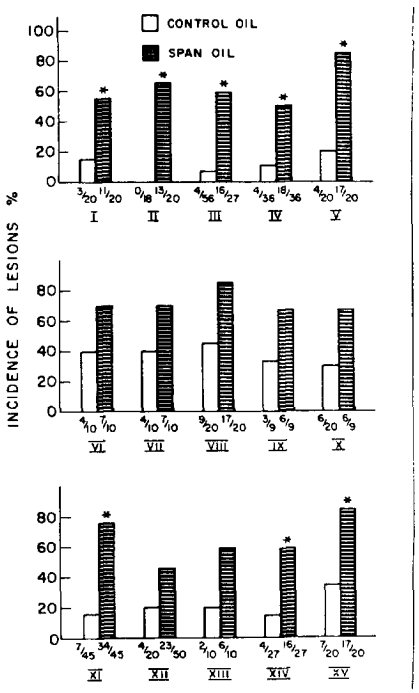

Fig. 3 Comparisons between Span oil and various control oils of effect on rat hearts

	Control oil	References
I	lard: corn	Beare-Rogers et al., 1974
II	lard: corn	ibid
III	lard: corn	ibid
IV	lard: corn	ibid
V	lard: corn	ibid
VI	corn	Kramer et al., 1973
VII	corn	Charlton et al., 1975
VIII	corn	Kramer et al., 1975b
IX	corn	Hulan et al., 1977d
X	lard	Hulan et al., 1976c
XI	lard	ibid
XII	olive	Kramer et al., 1975b
XIII	olive	ibid
XIV	soybean	Beare-Rogers et al., 1974
XV	saffloer	Kramer et al., 1975b

*Treatment incidence significantly greater control incidence at 5% level by the Fisher-Irwin test (Fleiss, 1973).

linolenic acid, however, could not be so characterized (BEARE-ROGERS & NERA, 1977).

In these studies, the test oil constituted 40% of the total energy of the diet. No matter how little or how much the animal consumes, the proportional contribution from the oil remained the same. If instead of concentrating on a source of energy, it would be possible to consider that the rat was being used to assay a non-nutrient component of the oil, the level of dose relative

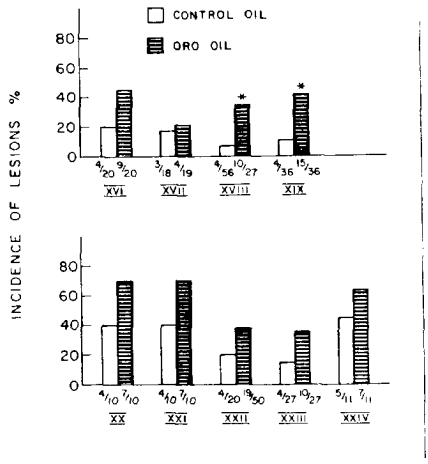

Fig. 4. Comparisons between Oro oil and various control oils of effect on rat hearts

	Control oil	Reference
XVI	lard: corn	Beare-Rogers et al., 1974
XVII	lard: corn	ibid
XVIII	lard: corn	ibid
XIX	lard: corn	ibid
XX	corn	Kramer et al., 1973
XXI	corn	Charleton et al., 1975
XXII	olive	Kramer et al., 1975b
XXIII	soybean	Beare-Rogers et al., 1974
XXIV	soybean	Lall et al., 1977

to body weight would be of prime consideration. In that light, the 2 grams of fat consumed in a day by a 50 g rat would be equivalent to 2 kilograms of fat in a 50 kilogram young growing man. This would indeed be an unreasonable intake of fat.

So far no cardiotoxic component beside erucic acid has been shown to be present in rapeseed oil. Unlike other rats tested, hooded rats lacking a lymphatic system did not develop cardiac lesions after prolonged ingestion of rapeseed oil (HULAN et al., 1977a). All of the absorbed fat transported by the portal system could therefore be subjected to liver methabolism, including detoxification. The benefits derived from hydrogenation also lack a satisfactory explanation. Reduction of linolenic acid alone did not suffice, but further hydrogenation improved the oil. A change in a minor component might also have occurred.

The susceptibility of the male rat to cardiac lesions has been a matter of intense interest and controversy. Background lesions, whether caused by a high fat diet or other factors, are more apparent in some rat populations than in others. An example of fairly resistant rats is shown in Table 3. No lesions were detected in the control group. Blends of Tower oil with the control mixture or other vegetable oils, or Tower oil by itself, with or without added vitamin E, did not significantly increase the incidence of cardiac lesions.

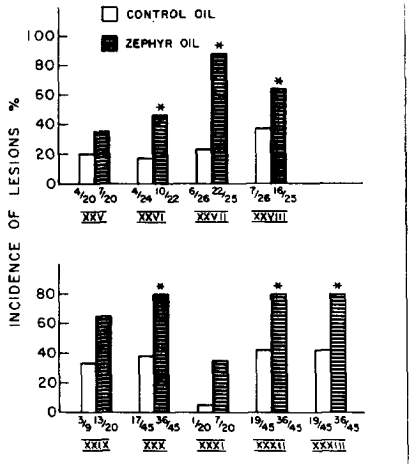

Fig. 5 Comparisons between Zephyr oil and various control oils of effect on rat hearts

	Control oil	Reference
XXV	lard: corn	Beare-Rogers et al., 1974
XXVI	corn	Hulan et al., 1977a
XXVII	corn	Hulan et al., 1977a
XXVIII	corn	Hulan et al., 1977c
XXIX	corn	Hulan et al., 1977d
XXX	corn	Hulan et al., 1977b
XXXI	olive	Beare-Rogers et al., 1974
XXXII	olive	Hulan et al., 1977b
XXXIII	soybean	ibid

Table 3: Wistar rats fed tower oil with or without other oils

Dietary fat	Cardiac Lesions			
	1	2	3	Total
	0	0	0	0/18
Control (lard: corn oil	2	1	0	3/18
Tower/control	1	1	0	2/17
Tower/palm/sunflower	0	1	0	1/18
Tower	3	0	1	4/18
Tower + Vitamin E				

There were alterations in the cardiac fatty acids that appear to be common for rats fed rapeseed oil. As shown in Figure 7, the C22:6 3 fatty acids replaced most of the C22:5ω6, particularly in the phosphatidyl ethanolamine. Since the C22:6ω3 is believed to arise from dietary linolenic acid, the effect of feeding linseed oil was investigated (BEARE-ROGERS et al., 1977). Its greatest effect on cardiac fatty acids was to increase the C22:5ω3, unlike rapeseed oil which, with a lower concentration of the linolenic acid, enchanced the C22:6ω3.

ESSENTIAL FATTY ACIDS

Rats fed various rapeseed oils were found to show symptoms similar to those described in essential fatty acid deficiency: alopecia, scaly tails and feet, hemorrhagic and necrotic tails (HULAN et al., 1976a). The condition, however, was most severe between 5 to 8 weeks, and disappeared after 14

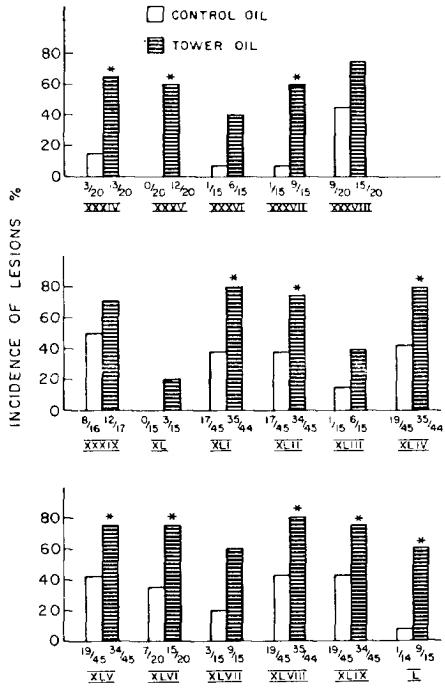

Fig. 6 Comparisons between Tower oil and various control oils

	Control oil	Reference
XXXIV	lard: corn	Beare-Rogers et al., 1977
XXXV	lard: corn	ibid
XXXVI	lard: corn	Beare-Rogers et al., 1978
XXXVII	lard: corn	ibid
XXXVIII	corn	Kramer et al., 1975b
XXXIX	corn	Slinger, 1977
XL	corn	Hung et al., 1977
XLI	corn	Hulan et al., 1977b
XLII	corn	ibid
XLIII	olive	Beare-Rogers et al., 1978
XLIV	olive	Hulan et al., 1977b
XLV	olive	ibid
XLVI	safflower	Kramer et al., 1975b
XLVII	sunflower	Beare-Rogers et al., 1978
XLVIII	soybean	Hulan et al., 1977b
XLIX	soybean	ibid
L	poppyseed	Beare-Rogers et al., 1978

weeks of the dietary treatment. Subsequently, skin from rats fed Zephyr or Span rapeseed oil or lard with erucic acid for 8 weeks exhibited a low capacity to synthesize prostaglandin E_2 (HULAN & KRAMER, 1977a). A correlation was noted between the dietary linoleate and the prostaglandin synthesis. Excess hair loss also occurred in rats fed heated rapeseed oil or heated lard (GABRIEL et al., 1976).

Partial hydrogenation of low erucic acid rapeseed oil destroyed its linoleic acid, and yet improved the myocardial condition (BEARE-ROGERS and NERA, 1977). Therefore an essential fatty acid deficiency did not appear to be a cardiotoxic factor.

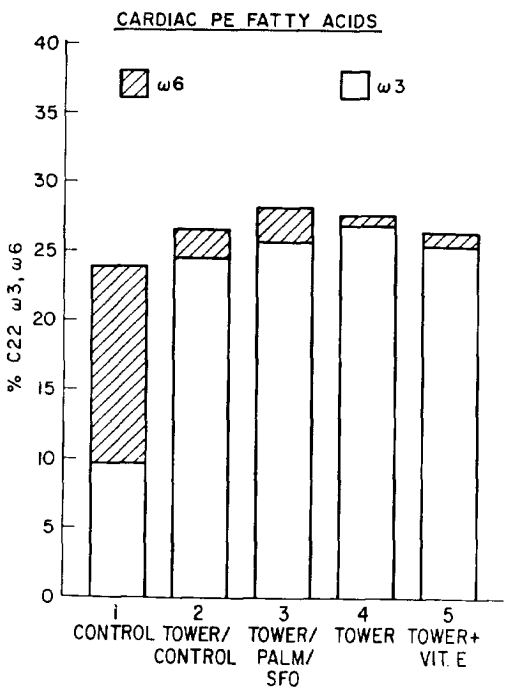

Fig. 7 C_{22} polyenoic fatty acids in cardiac phosphatidyl ethanolamine (PE)

OTHER MAMMALIAN SPECIES

Feeding trials with low erucic rapeseed oil for at least a year have yet to be done with a mammalian species other than the rat. For inter-species comparisons there is a need to consider an appropriate portion of the life-span of different animals.

With a rapeseed oil containing 40% erucic acid in the diet of the pig there was an early accumulation of erucic acid in heart and skeletal muscle and an increase in cardiac lipoprotein lipase (RAMPICHINI et al., 1976; SIMONETTI, 1976). Pigs fed high or low erucic acid rapeseed oil for periods similar to those used for rat studies (Table 4) did not exhibit any necrotic lesions that could be related to the dietary treatment (FRIEND et al., 1975 a, b; 1976; AHERNE et al., 1975, 1976). Studies at the ultrastructural level indicated that the mitochondrial volume gradually increased in pigs fed either low or high erucic rapeseed oils. After 60 days, intramitochondrial inclusions and mitochondrial deterioration were observed (VODOVAR et al., 1977). A feature of the membrane composition in the pig heart was that erucic acid was found to be high in alkenyl acyl-ethanolamine phosphoglyceride (KRAMER and HULAN, 1977).

Cynomolgus monkeys fed high erucic rapeseed oil still exhibited lipidosis in cardiac and skeletal muscles at 17 weeks but no evidence of cardiac necrosis that could be attributed to the diet (ACKMAN & LOEW, 1977; ACKMAN et al., 1977). After 24 weeks of feeding Tower oil (0.2% erucic acid) to cynomolgus monkeys, it was concluded that the rapeseed oil was indistinguishable from soybean oil in its nutritional and pathological properties (KRAMER et al., 1978 a,b).

PROTEIN CONCENTRATE

There have been significant developments during the last four years in evaluating the nutritional properties of the protein concentrates from rapesed. A proximate composition of low erucic seed, based on the average value from EL NOCKRASHY et al. (1975), is given in Table 4.

Table 4: Composition of low erucic rapeseed

	%
Oil	44
Protein (N X 6.25)	24
Ash	4
Fibre	9
Nitrogen-free extract	19
	100

The protein-rich fraction, free from most of the hulls, oil, water-soluble carbohydrates and glucosinolates of rapeseed, exhibited a high biological protein value (BELL et al., 1976); McDONALD et al., 1978). The amino acid spectrum showed that the rapeseed product contained more cystine and methionine than did soya protein concentrate (McLAUGHLAN et al., 1978). Nutritional concerns revolved around the non-protein constituents, particularly goitrogenic materials and chelating substances.

With the reduction in the glucosinolate levels of the protein concentrates there was an alleviation of antithyroid effects (LOEW et al., 1976). The toxic symptoms in the pregnant rat fed rapeseed protein concentrate were first described in Sweden (EKLUND, 1973). In retrospect, it is interesting to read that because the adverse effects were found in rats, but not in mice, the question was raised about merits of using the susceptible species for testing a plant protein (SHARPE et al., 1975).

Subsequently, in the rat, rapeseed protein concentrate was shown to be high in phytate (5.3 to 7.5%) and to precipitate a zinc deficiency (McLAUGHLAN et al., 1975). The condition was typified by a rapid loss in weight at 16 to 18 days of gestation, a low level of zinc in the blood serum and a high incidence of stillbirths. In weanling rats fed rapeseed protein concentrate, low levels of serum zinc were observed (SHAH et al., 1976; ANDERSON et al., 1976). Supplementation of the drinking water with zinc permitted weight gains and zinc concentrations in serum, liver and femur to reach those of the controls (SHAH et al., 1976). It was also demonstrated that to obtain a realistic value for the biological value of the protein, it was necessary to supplement a conventional salt mixture with zinc (McLAUGLAN et al., 1977).

The cause of the zinc deficiency is still speculative. The phytic acid was reported not to be the agent responsible in rapeseed protein concentrate for the symptoms repcipitated in the pregnant rat (LIEDEN & HAMBRAEUS, 1977). Also, neither phytate nor phenolic compounds affected protein utilization (McDONALD et al., 1978). There was evidence of low tocopherol levels in pregnant rats fed a rapeseed protein concentrate (EKLUND, 1975). That raised the question whether a factor was causing a vitamin E deficiency.

Supplemental zinc did overcome the anorexia, weight loss and stillbirths in the pregnant rat. As shown in Table 5 summarized from SHAH et al. (1978), the addition of zinc to drinking water (70 mg/1) permitted the rats receiving rapeseed protein concentrate to perform as well as those receiving casein.

Table 5: Body weight gains during pregnancy in rats fed rapeseed protein concentrate (RPC) (grams)

Time of pregnancy	Casein	RPC	RPC + Zn
wk.			
1	25	27	28
2	58	50	56
3	112	50	99
Live offspring pups/rat	11	8	11

It is apparent that both rapeseed oil and protein concentrate are still challenging the inquisitive researcher. It has been gratifying to view some light and to anticipate more illumination.

Dr. G. Jarvis provided statistical advice and analyses.

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