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Evaluation of Rapeseed Protein Concentrate as a Source of Protein in a Zinc Supplemented Diet for Young Rats

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In the first experiment 32 male Wistar weanling rats were divided into four equal groups on the basis of their body weights. The control group (D1) was fed 20% protein from casein and the other three groups received diets D2, D3, and D4 containing the same level of protein from Tower (*Brassica napus*) rapeseed protein concentrate (RPC). The amount of zinc added as $ZnSO_4$ to the four diets was 13, 100, 150, 200 μg/g, respectively. From the body weights and zinc levels in the liver and femur of the rats after a 4-week feeding period, the optimal level of zinc supplementation was found to be 150 μg/g. In the second experiment 48 weanling rats were allocated equally to three diet groups. The casein diets D5 and D6 contained 13 and 150 μg/g of zinc, respectively, and the RPC diet D7 had 150 μg/g of zinc. At the end of feeding for 8 and 16 weeks, there was no consistent and significant effect of feeding RPC on body weight, levels of zinc, iron, copper, manganese, and calcium in serum, liver, testes, and femur. Only the magnesium concentration in serum and femur was elevated but it did not result in any gross adverse effects. The thyroids of the RPC-fed rats were larger than those of the controls.

The optimal level of zinc supplementation of a diet for young rats, containing 20% protein from Tower (*Brassica napus*) rapeseed protein concentrate (RPC), was suggested to be 150–200 mg/kg diet (Shah et al., 1979). Using a 10% protein diet, Anjou et al. (1978) did not find any improvement in the PER (protein efficiency ratio) value for Span (*Brassica napus*) RPC by adding more than 70 mg/kg zinc to the diet of young male Sprague-Dawley rats. Moreover, they reported that the PER value (3.5) for RPC was much higher than other protein sources including textured soy flour, soy protein isolate, ground meat mixture, and casein. This level of zinc supplement is in general agreement with the above suggestion for a 20% protein diet. It is necessary, however, to determine the exact level of zinc supplement required in a rat diet containing 20% protein from RPC and to determine in a long-term study

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Table I. Weanling Rats Fed Diets Containing Casein or Tower RPC[a] for 4 Weeks

	casein	RPC		
diet no.	D1	D2	D3	D4
Zn added, μg/g	13	100	150	200
body weight, g	217 ± 12[b]	194 ± 20[c]	199 ± 18[c]	198 ± 17[c]
thyroids, mg	9.6 ± 0.8	9.7 ± 1.6	10.7 ± 1.9	11.1 ± 2.1
mg/100 g body weight	4.4 ± 0.4	5.1 ± 1.1	5.4 ± 1.0[d]	5.6 ± 1.0[d]
zinc, μg/g dry weight				
liver	83 ± 4	86 ± 5	83 ± 4	92 ± 6[d]
femur	262 ± 9	226 ± 12[c]	254 ± 11	276 ± 12[d]

[a] Rapeseed protein concentrate. [b] Mean ± standard deviation. Eight rats per group. [c] Significantly less than D1 ($P < 0.05$) by Student's t test (Freund et al., 1960). [d] Significantly higher than D1 ($P < 0.05$).

the effects on the metabolism of other mineral nutrients such as copper (Omole and Bowland, 1974) and iron (Anderson et al., 1976). Since RPC is high in phytate and phytate has also been shown to interfere with the metabolism of calcium and magnesium (Oberleas, 1973), it is essential to determine the effects on the skeletal metabolism of animals fed RPC for an extended period of time. The present investigation was planned for the nutritional assessment of RPC from these viewpoints.

MATERIALS AND METHODS

Animals, Diets, and Protocol. Experiment 1. To determine the optimal level of zinc supplementation, four diets including the control diet and three diets containing RPC with increasing levels of zinc were used. The control diet (D1) contained, in weight percent: casein, 23; sucrose, 20; corn starch, 44; vitamin mixture (Momcilovic et al., 1976), 1; salt mixture (Bernhart and Tomarelli, 1966), 3; corn oil, 5; cellulose type nonnutritive fiber, 4. The other three diets (D2, D3, and D4) had 34.5% Tower (*Brassica napus*) RPC providing 20% protein, and the same proportions of sucrose, vitamin, and salt mixtures as the control diet. Corn oil and nonnutritive fiber were adjusted to match the fat and fiber contents of the control diet in view of the composition of the RPC (Shah et al., 1979). Corn starch was added by difference. Zinc supplement, as zinc sulfate, was added so that the total added zinc (including that from the salt mixture) amounted to 100, 150, and 200 μg/g of diets D2, D3, and D4, respectively. The diets were fed ad libitum to male Wistar weanling rats (Canadian Breeding Farms, St. Constant, Quebec) for 4 weeks. Eight rats were allocated on body weight basis to each diet group according to a randomized block design. The average body weights of the groups were from 38.8 to 39.5 g. The animals were housed in individual stainless steel cages and they received distilled water for drinking. The body weight and food intake were recorded weekly.

At the end of 4 weeks the rats were killed by an overdose of "Euthansol" (sodium pentobarbital), and liver, thyroids, and the right femur were removed. Fresh weights were recorded and the tissues were stored at −20 °C.

The zinc content of the livers and femurs was determined by atomic absorption spectroscopy as described previously (Shah et al., 1979).

Experiment 2. The effects of feeding diets containing the optimum level of zinc as determined in the first experiment for 16 weeks were investigated. The composition of the diet D5 and D6 containing casein was similar to diet D1 in experiment 1 except that diet D6 had 150 μg/g of added zinc. Diet D7 containing RPC had the same level of added zinc as diet D6. Sixteen male Wistar weanling rats were allocated on the basis of body weight to each group in a randomized block design. The average body weights of the groups ranged from 39.0 to 39.3 g. All the animals received distilled water for drinking. After feeding the diets for 8 weeks, eight rats, selected at random from each diet group, were killed, and blood, thyroids, liver, testes, and the right femur were collected from each rat and handled as before.

The remaining animals were fed the same diets for a further 8 weeks and killed. The same tissues were collected from each animal and handled in the same way.

Mineral Analysis. Serum was analyzed for zinc, iron, copper, calcium, and magnesium; liver for zinc, iron, copper, and manganese; testes for zinc, iron and copper; femur for zinc, calcium and magnesium, by atomic absorption spectroscopy (Shah et al., 1979).

RESULTS AND DISCUSSION

The results of experiment 1 are summarized in Table I. The body weights of the rats fed diets containing RPC were less than that of the controls, in spite of a higher level of zinc supplement than used in an earlier experiment (Shah et al., 1979), although an increasing trend in body weight was observed with an increase in added zinc from 25 to 100 μg/g. The differences between the weights of the thyroids of the rats fed the control and the experimental diets were not significant. On equal body weight basis, however, the thyroids of rats fed RPC diets supplemented with 150 and 200 mg/kg of zinc were significantly larger than those of the control animals.

From the zinc levels in the liver, this difference in growth could not be attributed to a low zinc status. Moreover, the zinc content of the liver for group D4, which received a diet containing 200 μg/g of zinc, was significantly higher than the controls, indicating that there was no need to add to the diet more than 150 μg/g of zinc. Similar conclusions can be drawn from the zinc content of the femur. It should be note that, whereas the femur zinc content for group D2 (100 μg of zinc/g) was less than that for control group D1, there was no significant difference between the values for groups D1 and D3 (150 μg of zinc/g). Thus it was confirmed that the optimal level of zinc supplementation of a rat diet containing RPC was 150 μg/g.

As pointed out before (Shah et al., 1976), the slight growth depression at 4 weeks could be attributed to the presence of glucosinolates (total as *N*-butylisothiocyanate, less than 0.10 mg/g, oxazolidinethione, less than 0.10 mg/g) in the Tower RPC used in these experiments. In the case of Bronowski RPC, which was almost free from glucosinolates, zinc supplementation was shown to prevent growth depression at 4 weeks. Attempts to reduce further the glucosinolate content of Tower RPC are in progress.

The results of the second experiment are summarized in Tables II (8 weeks) and III (16 weeks). The difference in body weight, seen after a 4-week feeding period (Table I), was not found after feeding a zinc-supplemented (150 μg/g) diet containing RPC for 8 or 16 weeks. The reason for slight depression in body weight at 4 weeks could be the fact that it takes longer than 4 weeks for the rats to get used to diet containing 20% RPC. Palatability problems with rapeseed have been reported earlier (Seoane and

Table II. Weanling Rats Fed Diets Containing Casein or Tower RPC[a] for 8 Weeks

	casein		RPC
diet no.	D5	D6	D7
Zn added, μg/g	13	150	150
body weight, g	352 ± 25[b]	354 ± 36	332 ± 19
thyroids, mg	12.6 ± 1.0	11.4 ± 1.8	15.5 ± 2.0[c]
mg/100 g body weight	3.6 ± 0.5	3.2 ± 0.4	4.6 ± 0.7[c]
serum, μg/g			
zinc	1.5 ± 0.6	1.4 ± 0.4	1.7 ± 0.9
copper	1.1 ± 0.5	1.0 ± 0.3	1.0 ± 0.2
calcium	102 ± 18	81 ± 20[e]	107 ± 17[d]
magnesium	19 ± 4	14 ± 3[e]	25 ± 7
liver, μg/g dry weight			
zinc	76.6 ± 6.4	78.1 ± 11.5	85.1 ± 8.6
iron	180 ± 27	176 ± 23	147 ± 34[e]
copper	12.7 ± 1.0	12.1 ± 1.2	11.8 ± 1.0
manganese	7.54 ± 0.45	7.61 ± 1.14	7.85 ± 0.78
testes, μg/g dry weight			
zinc	185 ± 9	194 ± 14	192 ± 6
iron	129 ± 14	114 ± 11[e]	95 ± 18[c]
copper	14.1 ± 0.4	13.9 ± 0.4	14.5 ± 0.4
femur, per g dry weight			
zinc, μg	287 ± 5	282 ± 11	277 ± 16
calcium, mg	232 ± 12	228 ± 8	224 ± 9
magnesium, mg	5.08 ± 0.34	5.02 ± 0.25	5.64 ± 0.28[c]

[a] Rapeseed protein concentrate. [b] Mean ± standard deviation. Eight rats per group. [c] Significantly different from D5 and D6 by Student's test, $p < 0.05$. [d] Significantly different from D6 $p < 0.05$. [e] Significantly different from D5 $p < 0.05$.

Table III. Weanling Rats Fed Diets Containing Casein or Tower RPC[a] for 16 Weeks

	casein		RPC
diet no.	D5	D6	D7
Zn added, μg/g	13	150	150
body weight, g	455 ± 41[b]	493 ± 31	471 ± 35
thyroids, mg	12.2 ± 1.5	13.7 ± 2.7	17.8 ± 2.2[c]
mg/100 g body weight	2.7 ± 0.3	2.7 ± 0.5	3.8 ± 0.4[c]
serum, μg/g			
zinc	1.2 ± 0.4	1.8 ± 1.0	1.9 ± 0.9
copper	2.0 ± 1.3	1.2 ± 0.2	1.4 ± 0.4
calcium	88 ± 34	100 ± 16	117 ± 25
magnesium	18 ± 8	19 ± 4	29 ± 8
liver, μg/g dry weight			
zinc	81.9 ± 5.7	77.0 ± 5.2	85.7 ± 2.8
iron	246 ± 48	219 ± 27	242 ± 36
copper	13.5 ± 1.1	12.7 ± 0.8	12.1 ± 0.5
manganese	7.08 ± 1.30	8.00 ± 0.65	7.53 ± 0.55
testes, μg/g dry weight			
zinc	189 ± 7	189 ± 9	188 ± 13
iron	188 ± 16	187 ± 13	183 ± 31
copper	15.6 ± 1.1	15.1 ± 0.5	15.9 ± 1.9
femur, per g dry weight			
zinc, μg	283 ± 17	291 ± 19	290 ± 17
calcium, mg	239 ± 9	232 ± 5	234 ± 4
magnesium, mg	4.66 ± 0.26	4.56 ± 0.21	5.11 ± 0.24[c]

[a] Rapeseed protein concentrate. [b] Mean ± standard deviation. Eight rats per group. [c] Significantly different from D5 and D6 by Student's t test, $p < 0.05$.

Gorrill, 1975). Food intake and food efficiency data (not given in the text) indicated that at 4 weeks the food intake of the rats fed RPC was less than that of the controls, but there was no difference in the food efficiency. At 8 and 16 weeks the difference in food intake was also eliminated. The enlargement of the thyroids persisted at 4, 8, and 16 weeks. The data reported by Eklund and Ågren (1974), who fed a diet containing 20% protein from RPC (Swedish variety of winter rape called "Panther") for 90 days, also showed a slight enlargement of thyroids in male Sprague-Dawley rats but not in females. On the other hand, Loew et al. (1976) reported that thyroid/100 g body weight was greater than the controls only in female Wistar rats fed 40% Echo or Tower rapeseed flour. In our experiment Wistar male rats showed definite thyroid enlargement. The sex related difference in the Sprague-Dawley rats is interesting but needs to be confirmed. The weights of liver, femur, and testes (data not given) were not affected by the diet containing RPC. Similar observations were reported by Eklund and Ågren (1974) and by Loew et al., (1976).

The zinc supplement of 150 μg/g of diet brought the serum zinc levels in RPC-fed rats up to normal. The only other consistent effect of feeding RPC was the increase in serum magnesium content at both 8 and 16 weeks. A similar increase in pregnant rats was observed before (McLaughlan et al., 1975). These levels, however, are much below the serum magnesium level of 100 μg/g at which central nervous depression begins to appear (Shils, 1974). The serum magnesium levels were consonant with the increase in the magnesium content of the femurs taken from the same rats at 8 and 16 weeks. It should be noted, however, that there was no consistent change in the serum or femur magnesium level from 8 to 16 weeks, probably indicating that these parameters had reached a plateau.

Although the liver copper level was found to decrease, as a result of RPC feeding for 4 weeks (Shah et al., 1979), a similar effect was not observed after a feeding period of 8 or 16 weeks. The liver manganese content was not affected, and the changes in the levels of zinc and iron were not consistent. Thus the adverse effect of dietary zinc on liver copper stores reported by O'Dell et al. (1976) in rats and by Omole and Bowland (1974) and Ivan and Grieve (1975) in Holstein calves was not observed by us, either in the rats fed casein or RPC with a zinc supplement of 150 μg/g diet, in the presence of about 6 μg/g of added copper. Anderson et al. (1976) suggested that feeding a zinc supplemented diet containing rapeseed flour had an adverse effect on the metabolism of iron and manganese. Although the level of iron in the liver and testes was less than the controls at 8 weeks, a similar difference was not seen at 16 weeks. There was no difference in the liver manganese stores at either time. This could indeed be explained by the observation of Ivan and Grieve (1976), that zinc supplementation did not affect net absorption of manganese in Holstein calves.

The changes in zinc, iron, and copper levels in the testes of the rats, due to RPC feeding, were not consistent. The slight adverse effect of dietary zinc on the copper content of the testes reported by O'Dell et al. (1976) was also not observed by us.

From 8 to 16 weeks the iron levels in liver and testes increased significantly in all the groups and the copper content of the testes showed a similar trend. Interestingly, the magnesium content of the femur decreased slightly but significantly from 8 to 16 weeks, whereas the levels of zinc and calcium did not change appreciably.

Zinc supplementation of the diet containing casein affected calcium and magnesium level in serum and iron in testes at 8 weeks, but at 16 weeks no significant differences were noted.

Thus a zinc supplement of 150 μg/g to a rat diet containing 20% protein from RPC was sufficient to completely overcome the symptoms of zinc deficiency and did not have any adverse effects on the tissue levels of zinc, iron, copper, manganese, calcium, and magnesium. Whether the enlargement of thyroids would persist when Tower RPC with reduced glucosinolates is included in the diet remains to be determined.

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Compounds Produced by the Reaction of 2-Hydroxy-3-methyl-2-cyclopenten-1-one with Ammonia and Hydrogen Sulfide

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The compounds produced from three model browning systems [2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene)/NH_3, cyclotene/H_2S, and cyclotene/NH_3/H_2S] were isolated and identified. The main constituents of these model browning systems were sulfur- and nitrogen-containing heterocyclic compounds, which included a thiol, pyrazines, cyclic ketones, and cyclic methylene polysulfides.

The browning reactions play an important role in determining the acceptance of processed and stored foods (Lea, 1950; Shallenberger et al., 1959; Pomeranz et al., 1962). Compounds formed during heat treatment of food constituents have been determined either using browning model systems to elucidate the formation mechanisms (Rizzi, 1972, 1974; Shibamoto and Bernhard, 1977) or through analyses of cooked foods to determine the conditions of formation (Stoll et al., 1967; Watanabe and Sato, 1971; Walradt et al., 1971). The browning reactions produce tremendous numbers of chemicals which range from highly volatile compounds (e.g., formaldehyde, methanol,

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