Optimal Level of Zinc Supplementation for Young Rats Fed Rapeseed Protein Concentrate

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To determine the optimal level of zinc supplement required by young rats fed rapeseed protein concentrate (RPC) and to investigate the mineral interactions, one group of ten male Wistar weanling rats was fed a 20% casein diet for 4 weeks and three other groups were fed the same level of protein from Tower (*Brassica napus*) RPC with a zinc supplement of 25, 50, or 100 mg/kg diet. The rats fed RPC gained 10–20% less weight than the controls, but there was no significant difference in thyroid weights. The concentrations of zinc in serum and femur were lower in the rats fed RPC, but the zinc concentration in the liver was higher. Serum copper was normal. Serum cholesterol was 25% lower in all the experimental groups in spite of the higher Zn/Cu ratios of the minerals added to the diet and found in the liver. From the serum and femur zinc levels it is estimated that a zinc supplement of 150 to 200 mg/kg diet would be required for young rats fed RPC to overcome zinc deficiency.

Zinc supplementation through drinking water containing 70 mg of zinc/L (as $ZnSO_4$) was shown to have a beneficial effect in young rats fed diets containing protein concentrates from rapeseed or mustard (Shah et al., 1976). Similarly, the toxic effects shown by pregnant rats fed Tower rapeseed protein concentrate (RPC) were almost completely eliminated by the same level of supplementary zinc (Shah et al., 1978). A zinc supplement of 300 mg/kg diet was, however, found by Anderson et al. (1976) to be necessary to prevent a decrease in food consumption, weight gains, and plasma zinc levels in weanling rats fed rapeseed flour. They also reported a decrease in the plasma iron of these rats which could be attributed to rapeseed flour since it was independent of the level of dietary zinc. Such an effect on serum iron was not observed in RPC-fed rats after parturition (McLaughlan et al., 1975). Moreover, the effect of increased zinc intake on tissue stores of zinc and other trace elements needs to be investigated especially in view of the antagonism between zinc and copper reported by Omole and Bowland (1974) in pigs, by Van Campen (1969) in rats, and by Ivan and Grieve (1974) in Holstein calves. It has been suggested on the basis of observations in rats and ruminants that zinc and copper interact through a common binding protein in the liver (Davies, 1974). Since an increased ratio between dietary zinc and copper has been implicated in hypercholesterolemia in rats (Klevay, 1973), it is necessary to determine the minimum level of zinc supplementation required to prevent deficiency in animals fed diets containing RPC.

Dietary phytate has an adverse effect on the availability of zinc if the molar ratio between phytic acid and zinc exceeds 30 (Quarterman, 1973). This ratio in RPC is approximately 47 since RPC contains about 5.7% phytic acid and 120 mg/kg of zinc (Shah et al., 1978). In order to bring this ratio down to 30, a dietary zinc supplement of about 70 mg/kg would be required.

In view of these considerations, the present investigation was undertaken to determine the optimal level of zinc supplementation of a rat diet containing 20% protein from RPC and also to study its effect on tissue stores of other trace elements, especially copper, and to determine the effect on serum cholesterol.

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Table I. Composition of the Diets (g/100 g)

	casein	RPC[a]
protein source	23.0	34.5
sucrose	20.0	20.0
corn starch	44.0	37.9
vitamin mixture[b]	1.0	1.0
salt mixture[c]	3.0	3.0
corn oil	5.0	1.7
nonnutritive fiber	4.0	1.9

[a] Rapeseed protein concentrate containing: g/100 g dry weight, crude protein, 60.8; fat, 10.1; crude fiber, 6.3; ash, 7.3; phytate phosphorus, 1.61; total phosphorus, 2.41; calcium, 0.80; magnesium, 0.82. μg/g dry weight, iron, 133; zinc, 121; copper, 3. Glucosinolate, mg/g: total (as *N*-butyl isothiocyanate), less than 0.10; oxazolidinethione, less than 0.10. [b] Momcilovic et al. (1976). [c] Bernhart and Tomarelli (1966).

MATERIALS AND METHODS

Animals, Diets, and Protocol. Forty male weanling Wistar rats, obtained from Canadian Breeding Farms, St. Constance, Quebec, were distributed in a randomized block design among four equal groups. The average body weights of the groups ranged from 37.5 to 37.9 g. The animals were housed in individual stainless steel cages and were fed ad libitum the control diet (group 1) containing 20% protein from casein and groups 2, 3, and 4 were fed the test diets containing the same level of protein from Tower rapeseed (*Brassica napus*) protein concentrate and 25, 50 or 100 mg/kg of zinc from zinc sulfate (Table I). The fat and crude fiber contents of all the diets were equalized. All the rats 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 blood, thyroid, liver, and the right femur were removed. Serum was separated and analyzed for cholesterol using the Harleco kit (Gibbstown, N.J.). The remaining serum was stored at –20 °C until used for the analysis of mineral elements. Fresh weights of the thyroid, liver, and femur were recorded and the tissues were stored at –20 °C.

Mineral Analysis. Serum was mineralized with nitric and perchloric acids. Zinc, iron, copper, calcium, and magnesium in the extracts were determined by flame atomic absorption spectroscopy using a Varian Techtron AA5 instrument, equipped with automatic background correction and a Techtron AA6 digital readout module. The results were expressed as microgram/gram of serum.

The femurs and livers were dried in an oven at 105 °C and ashed overnight in a muffle furnace at 450 °C. Nitric

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Table II. Body and Organ Weights, Mineral Elements in Tissues and Serum Cholesterol at 4 Weeks

	casein	Tower RPC[a]		
Zn added, μg/g	13	25	50	100
body weight, g	202 ± 20^{a} [b]	170 ± 14^{b}	176 ± 11^{b}	182 ± 20^{b}
gain, g/100 g food	42 ± 2	39 ± 2	42 ± 2	43 ± 2
thyroids, mg	7.4 ± 0.8	7.9 ± 1.6	7.2 ± 1.0	8.5 ± 2.2
mg/100 g	3.7 ± 0.5^{a}	4.7 ± 1.0^{b}	4.1 ± 0.6^{a}	4.7 ± 1.3^{b}
liver dry weight, g	2.40 ± 0.32^{a}	2.02 ± 0.23^{b}	2.06 ± 0.25^{b}	2.07 ± 0.27^{b}
g/100 g	1.19 ± 0.12	1.18 ± 0.06	1.16 ± 0.11	1.13 ± 0.07
femur dry weight, mg	271 ± 20^{a}	231 ± 14^{b}	243 ± 19^{b}	247 ± 26^{b}
mg/100 g	134 ± 6	136 ± 6	138 ± 8	136 ± 7
serum, μg/g				
zinc	1.65 ± 0.25^{a} [b]	0.89 ± 0.28^{b}	1.10 ± 0.26^{b}	1.34 ± 0.30^{b}
iron	3.61 ± 1.03	2.50 ± 1.23	3.35 ± 0.75	2.29 ± 1.10
copper	0.76 ± 0.10	0.72 ± 0.05	0.78 ± 0.09	0.78 ± 0.05
calcium	132 ± 8^{a}	125 ± 4^{b}	129 ± 4^{a}	126 ± 2^{b}
magnesium	20 ± 3	21 ± 2	22 ± 2	22 ± 3
liver, μg/g dry weight				
zinc	78 ± 2^{a} [b]	83 ± 4^{b}	82 ± 5^{b}	84 ± 5^{b}
iron	117 ± 16	115 ± 18	123 ± 25	114 ± 15
copper	21 ± 5^{a}	15 ± 1^{b}	15 ± 2^{b}	16 ± 4^{b}
femur, μg/g dry weight				
zinc	266 ± 12^{a} [b]	114 ± 14^{b}	154 ± 19^{b}	217 ± 21^{b}
calcium × 10^{-3}	267 ± 4	263 ± 6	263 ± 6	261 ± 6
magnesium × 10^{-3}	3.78 ± 0.18^{a}	4.18 ± 0.22^{b}	4.25 ± 0.11^{b}	4.17 ± 0.17^{b}
serum cholesterol, mg/dL	126 ± 32^{a} [b]	94 ± 17^{b}	101 ± 31^{b}	98 ± 24^{b}
Zn (μg/g)/Cu (μg/g)				
serum	2.9 ± 0.3^{a}	1.2 ± 0.4^{b}	1.4 ± 0.4^{b}	1.7 ± 0.4^{b}
liver	3.6 ± 1.0^{a}	5.6 ± 0.4^{b}	5.7 ± 0.6^{b}	5.4 ± 1.1^{b}
diet (added Zn & Cu)	2.6	5.1	10.2	20.5

[a] Rapeseed protein concentrate. [b] Mean ± standard deviation. Ten rats/group. Values, in a row, having different superscript letters are significantly different, $P < 0.05$.

acid was used to complete ashing. The ash was dissolved in 25% (v/v) concentrated hydrochloric acid, and the following elements were determined by atomic absorption spectroscopy: zinc, iron, and copper in liver; zinc, calcium, and magnesium in femur. The results were expressed in terms of microgram/gram dry weight.

Statistics. A two-way analysis of variance appropriate to the random block design was carried out on all the variables according to Cochran and Snedecor (1973). A significance level of 0.05 was used. If the analysis of variance indicated a real difference between treatment means for any variable, it was further analyzed by Dunnett's test (Miller, 1966), which compared the means for diets 2, 3, and 4 with that for the control diet individually. Again a significance level of 0.05 was used for these comparisons.

RESULTS AND DISCUSSION

The data on body and organ weights, food efficiency, mineral elements in tissues, and serum cholesterol at 4 weeks are summarized in Table II. The mean body weight of the controls at the end of the 4 weeks was higher than the means for the three test diet groups. Although the average body weights of the RPC-fed groups increased with increasing zinc level in the diet, the trend was not significant. Anderson et al. (1976) also did not find a significant increase in the 4-week body weight gain of rapeseed flour-fed Wistar rats even when 150 mg/kg of zinc was added to the diet. On the other hand, Eklund et al. (1974) fed young male and female Sprague-Dawley rats with diets containing a Swedish rapeseed protein concentrate (' Panther ' variety of winter rape, *Brassica napus* L., var. Olifiera D.C.) for 12 weeks without any growth inhibition during the entire period, although the salt mixture provided only 21 mg/kg of zinc. Some differences in the observations may be attributable to the variation in the phytic acid content, the glucosinolate content of the rapeseed product used, or unknown sources of zinc. For example, the phytic acid phosphorus content of the rapeseed flour investigated by Anderson et al. (1976) was about 1.2% as compared to 1.6% in the RPC used in this work. Eklund et al. (1974), however, did not report the phytate content of their product. The rapeseed flour used by Anderson et al. (1976) was free from oxazolidinethione but it contained 0.005 mg/g of isothiocyanates, and the RPC in the diets fed by Eklund et al. (1974) was glucosinolate free. The RPC investigated by us contained less than 0.10% of glucosinolates. The experimental animals were housed by the former group and most probably by the latter group (Eklund and Ågren, 1978) in galvanized cages, which can be a significant source of zinc (National Research Council, 1972; Stevens et al., 1977). Moreover, tap water containing 3 mg/L of zinc was used by Anderson et al. (1976), whereas Eklund et al. (1974) did not specify the drinking water used in their work.

The body weight gain per 100 g of food consumed did not appear to vary appreciably between the control and the test diet groups. This may be interpreted to mean that for some unknown reason the food intake of the rats which received the test diets was slightly depressed, since their body weights were lower. Moderate zinc deficiency as indicated by serum levels is one of the factors responsible for depressed appetite and hence food intake (Underwood, 1977).

The weight of the thyroids was not significantly affected by the test diets, but on equal body weight basis, the animals in groups 2 and 4 had larger thyroids. Eklund et al. (1974) also did not observe any enlargement of thyroids in young male and female rats after feeding RPC diets for 12 weeks. A similar observation was reported by Anderson et al. (1976) in pregnant rats fed rapeseed flour. Thus, by plant breeding and by the processing involved in the preparation of rapeseed protein concentrates, the antithyroid substances have been reduced to very low levels.

The liver and femur weights for the test diet groups were lower than those for the casein-fed animals. When expressed on equal body weight basis, however, these differences were eliminated. In their 12-week study, Eklund et al. (1974) did not find any effect of RPC feeding on liver weights, just as they did not observe any differences in body weight gains.

The mineral elements in the serum indicate that serum zinc in the test groups was 20–45% lower than the level in the controls but it increased with increasing dietary zinc. Thus, there is a need for a higher level of zinc supplementation of diets containing RPC. Besides observing a similar decrease in plasma zinc levels in rapeseed flour fed rats, Anderson et al. (1976) also noted a significant decrease in plasma iron. In this study, however, not only was serum iron unaffected, but serum levels of copper and magnesium did not change. Similar results were reported in pregnant rats (McLaughlan et al., 1975). The changes in serum calcium were not consistent.

There was a small increase in the concentration (but not in the amount) of zinc in the liver of the test groups but the level of liver copper and the total amount showed a decrease in the RPC-fed rats. There was no effect on the iron content of the liver. Since the increase in dietary zinc did not result in a corresponding decrease in liver copper, the antagonism between zinc and copper (Davies, 1974; O'Dell et al., 1976) could not be implicated. However, it remains to be ascertained if further increases in the dietary zinc level will cause decreases in liver copper.

The mineral analysis of the femur also indicated lower zinc levels in the test groups. With increasing dietary zinc supplement, femur zinc increased, but even with 100 mg/kg of zinc the femur level of the element was about 20% less than that in the control group. This confirmed the need for a higher zinc supplement. Whereas femur calcium was not affected by RPC feeding, femur magnesium was significantly higher in the test diet groups than in the casein-fed rats. This may be due to the additional magnesium provided by the RPC (Table I) but a similar effect of RPC calcium was not seen on femur calcium. The lack of effect on femur calcium may be because of the poor availability of RPC calcium which is tightly bound to phytic acid (Reinhold, 1975).

The data on serum cholesterol, dietary ratio of added Zn/Cu, and the ratio in the tissues are also given in Table II. The ratio of zinc and copper added to the diet increased from 5.1 to 20.4 in the test diets but a similar change was not seen in either serum or liver. In spite of higher dietary Zn/Cu ratios, the serum cholesterol in all the test groups was about 20% less than in the controls. Although the crude fiber content of the diets was equalized, the control diet had nonnutritive cellulose, whereas in the test diets the crude fiber was made up of approximately 50% nonnutritive cellulose and 50% crude fiber from the RPC. Moreover, the test diets contained about 0.5% of phytin phosphorus which could have bound some of the added zinc. The hypocholesterolemic effect of fiber and phytâte (Klevay, 1977) was so predominant that the hypercholesterolemic effect of increasing dietary Zn/Cu ratio may not have been seen. The serum zinc levels in the test diet groups and hence the Zn/Cu ratio indicated suboptimal zinc status, which has been shown by Patel et al. (1975) to lower serum cholesterol. Thus, a decrease in serum zinc and a decrease in liver copper were associated with a lower serum cholesterol level.

Based on linear regression (Cochran and Snedecor, 1973) of the data on serum zinc and on femur zinc, it is estimated that a dietary zinc supplement of 150 to 200 mg/kg would be required to make the response of the Tower RPC-fed rats equivalent to those of the controls. Further experiments to confirm this prediction are in progress.

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