ANTINUTRITIONAL COMPONENTS IN RAPESEED AND THEIR EFFECT ON PREGNANT RATS

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INTRODUCTION

Studies on rapeseed protein concentrate have shown that it has very high NPU- and PER-values and no antinutritional effects were recorded on the growing rats (McDonald et al. 1978, Anjou et al. 1978).

In 1973 Eklund reported that toxic symptoms and a high fetal mortality were observed in pregnant rats given a diet containing a rapeseed protein concentrate with a low glucosinolate content. The symptoms were later further characterized and it was shown that there were no teratological effects on the puppies on the 18th day of gestation (Eklund & Ågren 1974, Sharpe et al. 1975). The severity of the effects could vary in different trials but it was found that one of the first effects on the pregnant rats was anorexia (lack of appetite) and a weight loss during the last days of the pregnancy. It was also shown that this effect was not caused by the glucosinolates.

In order to find out if there was an unknown antinutritional component in rapeseed and if it could be eliminated, a series of screening studies on pregnant rats were performed. While these studies were going on, it was suggested that phytic acid in rapeseed protein concentrate caused zinc deficiency, which in turn caused the symptoms in the animals (McLaughlan et al. 1975). The rat studies were then designed to put more light on the effect of phytic acid.

MATERIALS AND METHODS

The standard rapeseed protein concentrate (RPC) was prepared by the process described by Anjou et al. (1974) giving a glucosinolate content of only 0.1 mg/g. RPC's with reduced phytic acid content were prepared by using acid leaching steps in the process or using rapeseed grown without phosphorous fertilization. Some of these RPC's were also extracted a few times with 80% acetone and pure acetone. Rapeseed protein isolate was prepared by solubilization at pH 11.5 and precipitation at pH 6 (Gillberg and Törnell 1976).

The nitriles tested were prepared by autolyzing defatted rapeseeds and extracting them with acetone. After some prepurifying the nitriles were separated on Sephadex G-10 (Daxenbichler et al. 1968).

Other materials used in the tests were: cottonseed protein produced with the liquid cyclon process (LCP) (Vix et al. 1971), soyabean flour (textured, Supro 50, Ralston Purina Comp. USA), calcium phytate (Sigma, about 40%) and the controls: chow (ground commercial type pelleted diet R3, Anticimex, Sweden) and casein (sodium caseinate, DMV, Holland).

The female rats were given a standard diet with RPC or the other materials as the single protein source at 20% protein level. The added levels of Ca, Zn, Fe and Mg in the diet were 0.8%, 50 mg/kg, 325 mg/kg and 540 mg/kg, respectively. If standard RPC was used in the diet the total levels were: Ca 1.1%, Zn 90 mg/kg, Fe 375 mg/kg and Mg 1100 mg/kg.

The exact mating date was not recorded as four female rats were housed together with a male until the females showed signs of pregnancy, whereafter they were housed individually. From that time their weight and feed intake were recorded daily until parturition, which was assumed to be on day 22. For each diet the trial started with 8 female rats, but only those which really became pregnant could be used in calculation of the results.

In evaluating the experimental results the weight difference between day 19 and day 21 together with the diet consumption on day 21 were used as criteria for the effect caused by RPC. Duncan multiple range test was used to discriminate between the results for the different materials.

RESULTS AND DISCUSSION

Fig. 1 shows the results for pregnant rats given different protein sources in the diet. The standard RPC with a high phytic acid content (92 mg/g protein) caused, as found by others, anorexia with a low diet consumption and a weigh decrease the last days before parturition. If, however, the phytic acid in the rapeseed material was reduced, no effects were found. Of these materials the RPC prepared by acid leaching had a phytic acid content of 20 mg/g protein, the RPC from low phosphorous grown seeds 70 mg/g protein, and the rapeseed protein isolate (RPI) 3 mg/g protein.

For comparison cottonseed protein and soyabean flour were included. The first, with a high phytic acid content (95 mg/g protein), also caused anorexia but not so severe as RPC. Soyabean flour with a low phytic acid content showed no effect.

Casein with added calcium phytate to a phytic acid level of 87 mg/g protein gave no effect. Although too many conclusions should not be drawn from a single test, this indicates that other factors may be involved in causing the anorexia.

Fig. 2 shows that if the calcium content of a diet containing RPC is very low, the effect on the pregnant rats will disappear. This can be understood as it is known that phytic acid gives salts of very low solubility with zinc in the presence of excess of calcium (Oberleas 1966). This experiment gives very strong evidence for the explanation that it is the large amount of phytic acid in RPC that makes the zink unavailable, which in turn will cause the anorexia.

Fig. 3 shows how the anorexia can be overcome by an extra addition of 80 mg/kg Zn or 270 mg/kg Fe to the diet but not by adding 570 mg/g extra Mg. This also shows that too little zinc is available in a standard diet with RPC. It also indicates, however, that other metals may be involved in the complex with phytic acid.

In Fig. 4 an example can be seen of the fact that acetone extraction of RPC will not change its effect on pregnant rats. Again it shows that if a low level of calcium in the diet or RPC with a lower phytic acid content is used, no effect will be found.

Fig. 5 gives the results of adding 70 mg/kg to the chow diet of various nitriles, which are split products from the glucosinolates. In no case was anorexia or other effect found. A normal RPC usually has a nitrile content of <1 mg/kg, so it is beyond doubt that the nitriles in RPC cannot cause anorexia.

CONCLUSIONS

Although these trials are fairly simple, they clearly show the effect of anorexia of pregnant rats given RPC. Variations among the individual rats in the groups were sometimes very large. Even if significant differences were obtained between groups, the conclusions drawn from single results should not be regarded as final, as the number of animals in the groups was small. However, the overall results, including some more confirming results not given here, strongly indicate that it is the phytic acid in RPC that strongly binds zinc and makes it unavailable for the mother rat which has an increased demand of zinc in the last days of pregnancy. Moreover, all other more severe effects others have found on pregnant rats given RPC, have been described by Apgar (1968) on rats given a diet with a very low zinc level. The whole thing is, however, rather complicated and other factors may be involved in special cases, which also have been indicated by Liedén and Hambraeus (1977). Further, the results reported here show that the effect of other metals than zinc can be important. A different metal balance may be an explanation for the calcium phytate not causing any effect. It is possible that the anorexia will be seen only if the phytic acid is above a certain threshold level and this may differ depending on other factors. The results of this investigation also clearly show that there are ways to eliminate the negative effects on rats given RPC.

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Fig. 1: Growth study of pregnant rats given different protein sources in the diet

Protein source	Growth day 19-21 x), g		Diet consumption on day 21 x), g	
Chow	24.2	ab xx)	21	a xx)
Casein	23.3	ab	15	a
RPC (standard)	- 3.8	c	4	b
RPC (phytic acid reduced)	35.6	a	18	a
RPC (low-P-seeds)	30.7	ab	19	a
RPI (acid precipitated)	31.0	ab	15	a
Cottonseed protein (LPC)	10.8	d	11	c
Soyabean flour	20.8	bd	18	a
Casein + calcium phytate	24.0	ab	16	ac

x) parturition on day 22
xx) values followed by the same letter do not differ significantly (p=0.05)

Fig. 2: Growth study of pregnant rats given RPC and different content of calcium in the diet

Protein source	% Ca added to the diet	Growth day 19-21 x) g		Diet consumption on day 21 x) g	
Casein	0.03	24.5	a xx)	16	a xx)
Casein	0.8	23.3	a	15	a
Casein	1.2	17.8	a	16	a
RPC	0.03	22.1	a	16	a
RPC	0.8	- 1.2	b	7	b
RPC	1.2	- 1.4	b	6	b

x) parturition on day 22
xx) values followed by the same letter do not differ significantly (p=0.05)

Fig. 3: Growth study of pregnant rats given RPC with extra amount of metals added

Protein source	Extra amount of metal added to the diet, mg/kg	Growth day 19-21 x), g		Diet consumption on day 21 x), g	
Casein		23.3	a xx)	15	a xx)
RPC		- 3.8	b	4	b
RPC	Zn 80	22.8	a	14	a
RPC	Fe 270	18.8	a	14	a
RPC	Mg 570	6.2	b	8	b

x) parturition on day 22
xx) values followed by the same letter do not differ significantly (p=0.05)

Fig. 4: Growth study of pregnant rats given acetone extracted RPC

Protein source	% Ca added to the diet	Growth day 19-21x), g		Diet consumption on day 21 x), g	
RPC (acetone-extracted)	0.03	24.2	a xx)	15	a xx)
RPC (acetone-extracted)	0.8	- 0.8	b	7	b
RPC (low-P-seeds, acetone-extracted)	0.8	31.0	a	20	c

x) parturition on day 22
xx) values followed by the same letter do not differ significantly (p=0.05)

Fig. 5: Growth study of pregnant rats given nitriles (70 mg/g diet) which are split products from glucosinolates

Nitrile	Growth day 19-21 x), g		Diet consumption on day 21 x), g	
Control (Chow)	24.2	a xx)	21	a xx)
1-cyano-3-butene	28.3	a	21	a
1-cyano-4-pentene	27.7	a	22	a
1-cyano-2-hydroxy-3-butene	31.9	a	23	a
1-cyano-2-hydroxy-3,4-epitiobutane	26.8	a	21	a

x) parturition on day 22
xx) values followed by the same letter do not differ significantly (p=0.05)