VI. MEHL (FÜTTERUNG) / MEAL (FEEDING)
TOURTEAU (ALIMENTATION)

THE NUTRITIONAL VALUE OF LOW-GLUCOSINOLATE RAPESEED MEAL

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Introduction

The main gene source that is being used in plant breeding to decrease the glucosinolate content of rapeseed is the cultivar Bronowski, which has a low and genetically determined content of glucosinolates.

When the first small feeding experiments were carried out with Bronowski meal, produced from seed that was not heat-treated, it was found that the meal was significantly better as feed than a high-glucosinolate meal, which contained approximately 10 times as much glucosinolates as the Bronowski meal (Table 1). However, although the mice did grow on the Bronowski

Table 1: Weight gains of mice fed rapeseed meal with a high- (Rigo) or a low- (Bronowski) glucosinolate content

Expt. no.	Source of protein	Weight gain g/20 d
I	Rigo, no heat treatment	- 1.4 ± 0.2
	Bronowski, no heat treatment	+ 5.6 ± 0.4
II	Rigo, Meal from heat-treated seed	+ 8.9 ± 0.2
	Bronowski, Meal from heat-treated seed	+10.6 ± 0.2

meal, the weight gains were slow. A heat treatment of the seed greatly improved the feeding results of the low-glucosinolate meal as well as of the high-glucosinolate meal. The question arose whether the improvement in nutritional value by heat treatment of low-glucosinolate seed could be explained by myrosinase inactivation alone, or whether other detrimental factors were also involved. It also was regarded important to determine the optimal conditions of the heat treatment.

Methods

The growth studies were carried out, mainly according to MUNCK (1970), using 19-day-old specific pathogen-free NMRI male mice. Each diet was generally fed to 8 male mice, which were kept in separate cages. The feeding period was 11 days.

Glucosinolate content was determined according to the method of APPELQVIST and JOSEFSSON (1967) and is expressed by the quantities of isothiocyanates and oxazolidinethiones that may be released on hydrolysis by myrosinase. Autolysis products of glucosinolates were determined according to DAXENBICHLER et al. (1970). The method of SCHWIMMER (1961) was utilised for estimating myrosinase activity. The amino acid analyses were performed by Dr. D. Eaker, Institute of Biochemistry, University of Uppsala, Uppsala, as described by EAKER (1970), using a 55-cm column of Beckman AA-15 resin.

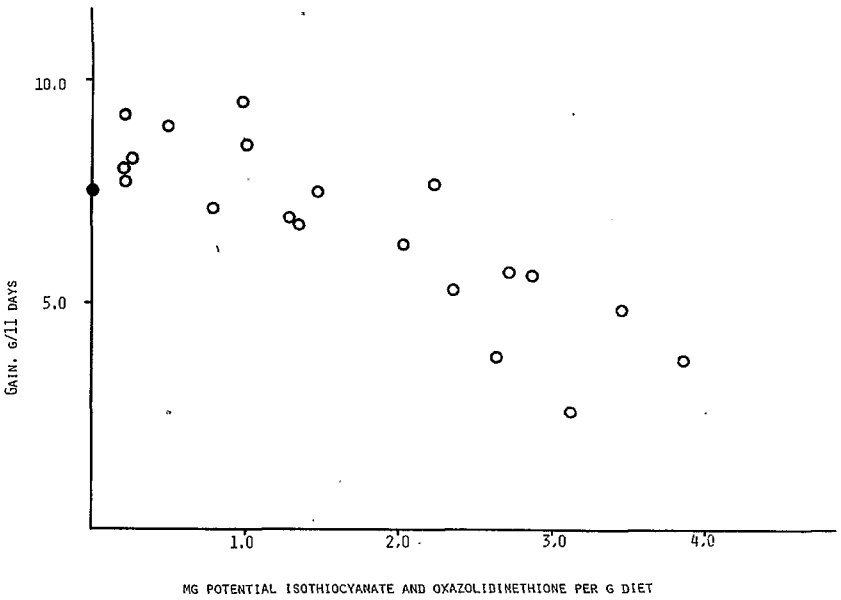
The heat treatments of meals used in the experiments presented in Table 5 were carried out with hot water. The other heat treatments were performed by heating seed in a closed vessel.

Nitriles were prepared as described by DAXENBICHLER et al. (1968).

Results and discussion

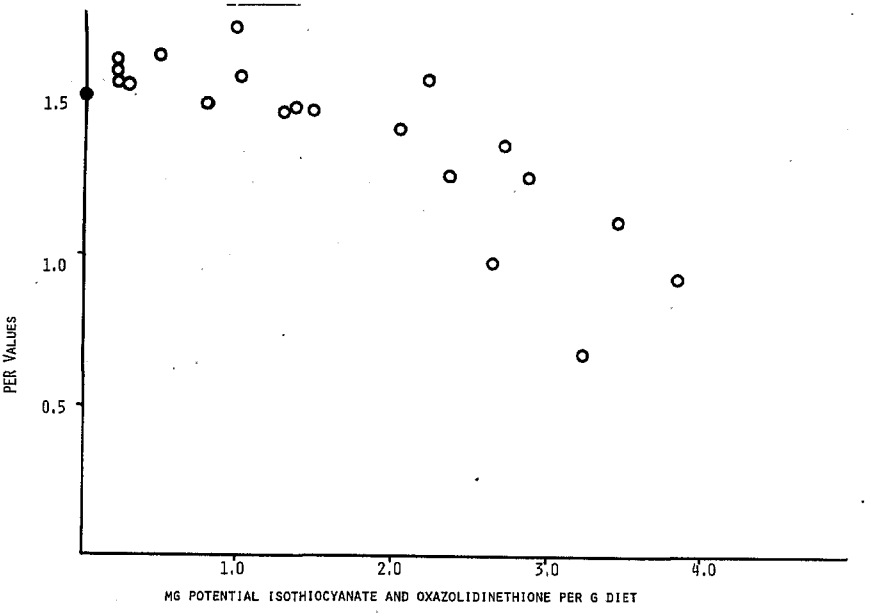
In order to study the influence of the glucosinolate content of myrosinase-inactivated seed on the nutritional value of the seed meal, feeding experiments were carried out using seed with a wide variation in glucosinolate content. In this experiment, as in all the experiments presented in this paper, rapeseed meal was the sole protein source and all the diets contained 10 % protein. The results of the experiments are shown in Figure 1. A glu-

Figure 1: Weight gains of mice in relation to releasable amounts of isothiocyanate and oxazolidinethiones in the diets



cosinolate content corresponding to a level of more than 1 mg potential isothiocyanates and oxazolidinethiones per gram diet depressed the 11-day growth of mice. Figure 2 shows that the protein efficiency ration (PER) were depressed by similar glucosinolate concentrations.

Figure 2: Values of PER in feed experiments with mice in relation to releasable amounts of isothiocyanates and oxazolidinethiones in the diets.



To determine the optimal conditions of the heat treatment of low-glucosinolate meal, the influence on the nutritional value of various moisture contents of the seed and of various temperatures and time lengths at the heat treatment were studied. The effects of the moisture contents when the seed was heat-treated at 90° C for 2 h are shown in Table 2. Obviously, myrosinase was not inactivated when the heat treatment was made at a 2 % moisture content. Further, the growth of the mice fed with meal from this seed was significantly slower than the growth of the other mice. There was some lysine destruction when the heat treatment was carried out at a 12 % moisture content. However, this did not influence the growth of the mice significantly, probably because the lysine content is not a limiting factor for the growth of mice when rapeseed meal is used as the sole protein source.

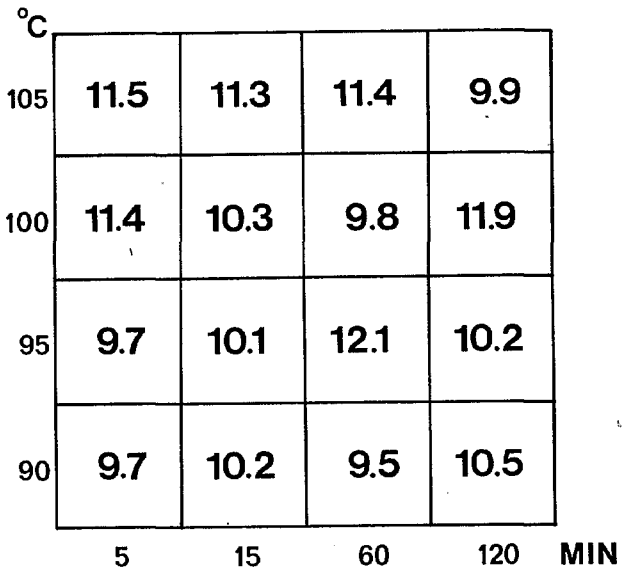
The influence of temperature and time length of the heat treatment was studied on seed with an 8 % moisture content. In the first experiment the

Table 2: Effect of heat treatment at various moisture contents on the nutritional value of rapeseed meal, cv. Bronowski

Moisture content of the seed at the heat treatment, %	Myrosinase activity, % of untreated Bronowski meal	Lysine, g/16 g N	Weight gain of mice, g/11 days	Feed intake (g)	PER value
Control (barley meal)	-	-	8.4 ± 0.4	49.8 ± 0.8	1.55 ± 0.05
2	100	6.18	5.3 ± 0.1	41.0 ± 0.9	1.11 ± 0.01
8	0	6.06	9.5 ± 0.2	48.8 ± 1.5	1.72 ± 0.01
12	0	5.39	8.5 ± 0.7	46.7 ± 0.9	1.63 ± 0.19
16	0	5.98	7.2 ± 0.6	44.0 ± 0.9	1.41 ± 0.11
24	0	6.20	9.5 ± 0.3	49.7 ± 0.7	1.60 ± 0.04
40	0	6.13	8.8 ± 0.7	47.4 ± 1.4	1.59 ± 0.09

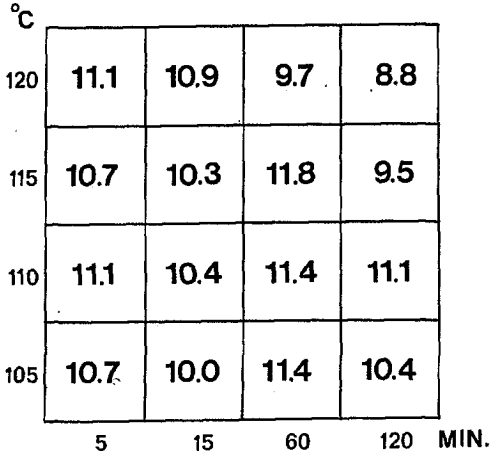
temperatures 90, 95, 100, and 105° C were tested with treatment times of 5, 15, 60, and 120 min at each temperature. Figure 3 indicates that there was a tendency toward better weight gains when the treatments were carried out at the higher temperatures. Thus, the possibility could not be dismissed that the optimum temperature might be higher than 105° C. For this reason,

Figure 3: Weight gains of mice fed rapeseed meals from seed of cv. Bronowski, heat-treated at 90-105° C and for various lengths of time



a second experiment was conducted in which meals were tested from seed heat-treated at 105, 110, 115, and 120° C. According to Figure 4, the treatment at 110° C was about as effective as the treatment at 105° C. The combination of higher temperatures and long treatment times resulted in lower weight gains.

Figure 4: Weight gains of mice fed rapeseed meals from seed of cv. Bronowski, heat-treated at 105-120° C and for various lengths of time



In order to investigate whether the growth-depressing effect of raw low-glucosinolate meal was caused by high- or low-molecular factors, a meal was extracted with water and the extract was dialysed. After freeze-drying, the sample was compared in a feeding test with a non-dialysed sample. As is shown in Table 3, weight gains and PER values were relatively high when the dialysed sample was used in the diet but not when the non-dialysed sample was used.

Table 4 shows how a seed meal prepared without heat treatment of the seed may be improved by dialysis. Adding the freeze-dried water that had been used in the dialysis to a casein diet resulted in equally great growth-depressing effects as when feeding a raw meal. Thus, the growth-inhibiting effects of a raw Bronowski meal seem to be neutralised by separating low-molecular compounds from high-molecular compounds by dialysis.

Since various hydrolysis products may be released from glucosinolates, a study of the growth-inhibiting effects of such compounds was deemed important. In the study presented in Figure 5, an oxazolidinethione preparation was added to a low-glucosinolate meal, prepared from heat-treated seed, and detoxified by ethanol extraction. The lowest amounts of

Table 3: Weight gains and PER values of mice fed proteins from rapeseed meal, cv. Bronowski

Protein source	Weight gain (g)	Feed intake (g)	PER value
Casein, supplemented with cysteine and methionine	13.9 ± 0.5	47.7 ± 0.8	2.47 ± 0.07
Meal, no heat treatment	0.6 ± 0.4	22.0 ± 0.9	0.36 ± 0.10
Meal from seed heat-treated at 90° C for 15 min	11.0 ± 0.6	49.4 ± 1.4	1.92 ± 0.08
Water-extract of meal, no heat treatment, freeze-dried	-0.2 ± 0.4	26.9 ± 1.0	- -
Water-extract of meal, no heat treatment, dialysed, freeze-dried	11.6 ± 0.6	45.4 ± 1.8	2.16 ± 0.08

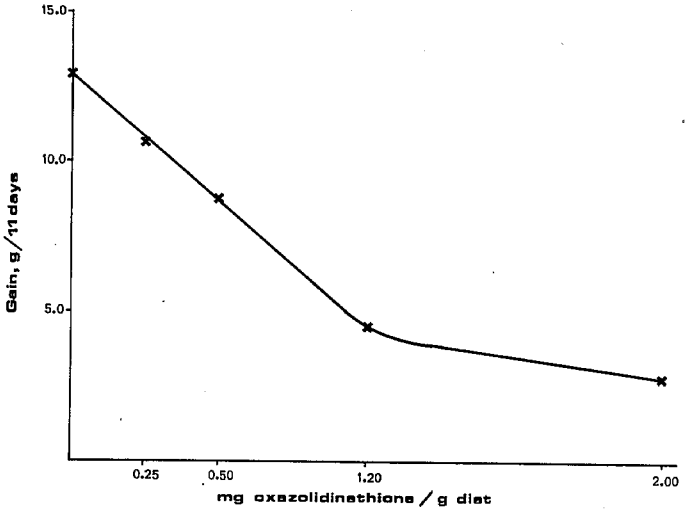
Table 4: Effect of dialysis on the nutritional value of rapeseed meal, cv. Bronowski

Protein source	Weight gain (g)	Feed intake (g)	PER value
Casein	14.8 ± 0.5	54.1 ± 1.2	2.32 ± 0.08
Meal, no heat treatment	1.9 ± 0.6	25.2 ± 1.7	0.68 ± 0.13
Meal, no heat treatment, dialysed, freeze-dried	11.4 ± 0.7	51.5 ± 2.6	1.88 ± 0.06
Casein with freeze-dried dialysis water added	1.7 ± 1.9	32.3 ± 3.8	- -

oxazolidinethione added corresponded approximately to the amount that may be released on complete hydrolysis of the glucosinolates in a Bronowski meal. A statistical analysis showed that this amount of oxazolidinethione significantly depressed weight gain and feed intake but not the PER values. On the other hand, the depression of weight gain was of a lower magnitude than that caused by a low-glucosinolate meal without heat treatment (Table 3).

Since glucosinolates hydrolysed to oxazolidinethiones and isothiocyanates do not seem to be responsible for the large growth-depressing effects induced by raw low-glucosinolate meal, the effects of hydrolysis to nitriles was studied. As has been shown by Van ETTEN et al. (1966), nitriles often are the predominant hydrolysis products of glucosinolates when a raw rapeseed meal is autolysed at room temperature. Table 5 presents results of feeding experiments with raw Bronowski meal and with three autolysed Bronowski meals. The raw meal, the autolysed and freeze-dried meal, as well as the autolysed, heat-treated and freeze-dried meal, resulted in

Figure 5: Effect of oxazolidinethione content on the nutritional value of rapeseed meal



similar- and low growth of mice. Thus, heat treatment after autolysis did not improve the nutritional value of the meal. On the other hand, extraction with methylene chloride removed the growth-depressing factors from the autolysed meal. Table 5 also discloses that these factors were left in the methylene chloride fraction after evaporation of the solvent. In earlier studies it has been shown that extraction of the meal with methylene chloride before autolysis did not result in the removal of any growth-depressing factor.

To study the influence of nitrile content on the nutritional value of rapeseed meal, various amounts of nitriles were added to a heat-treated and detoxified meal. The nitrile preparation, which contained 97 % nitriles, was prepared according to DAXENBICHLER et al. (1968) using Sephadex G-10 for purification. To the third sample in Table 6 about as much nitriles was added as is usually found on analysis of autolysed Bronowski meal. To the fifth sample the amount of nitriles was added that might be theoretically released in a Bronowski meal if the total amount of glucosinolates is converted into nitriles on autolysis. The fourth sample contained intermediate amounts of nitriles. The difference in weight gain between mice fed sample 1 and the sample with the smallest amount of nitriles was not statistically significant, which was in contrast to the difference in feed intake. The sample with intermediate amounts of nitriles was significantly inferior to sample 1 in promoting weight gain and feed intake as well as effecting an increase in the PER value. The mice fed with the sample with the highest nitrile content decreased in weight. A sample composed of 89 % of a heat-treated and detoxified meal and 11 % of a non-heat-treated,

Table 5: Influence of autolysis products on the nutritional value of low-glucosinolate rapeseed meal

Source of protein	mg/g diet			Myrosinase activity, % of untreated meal	Weight gain (g)	Feed intake (g)	PER value
	Nitriles	Oxazolidine-thiones	Isothio-cyanates				
Casein + 1.0 g methionine and 1.0 g cysteine per kg	-	-	-	-	15.6 ± 1.1	52.3 ± 1.6	2.41 ± 0.12
Meal, no heat treatment	0.18 *	0.16 *	0.08 *	100	3.1 ± 0.3	26.9 ± 1.0	0.95 ± 0.08
Meal, autolysed, freeze-dried	0.21	0.00	0.03	96	3.6 ± 0.3	26.6 ± 1.1	1.19 ± 0.08
Meal, autolysed, heat-treated, freeze-dried	0.14	0.00	0.03	0	3.2 ± 0.3	28.7 ± 0.7	0.95 ± 0.09
Meal, autolysed, extracted with CH_2Cl_2	0.06	0.00	0.03	88	11.9 ± 0.9	48.3 ± 2.7	2.06 ± 0.07
Casein with the substances extracted by CH_2Cl_2 added	0.17	0.00	0.00	-	0.2 ± 0.6	21.0 ± 1.2	- -

Each value is the average for 5 male mice ± S.E.

* After autolysis

Table 6: Effect of nitrile content on the nutritional value of rapeseed meal

Source of protein	Nitriles mg/g diet		Myrosinase activity, % of untreated meal	Weight gain (g)	Feed intake (g)	PER value
	Added	Found				
Casein + 1.0 g methionine and 1.0 g cysteine per kg	0.00	-	-	15.6 ± 1.1	52.3 ± 1.6	2.41 ± 0.12
Meal 1, Bronowski meal, heat-treated, extracted with 80 % ethanol	0.00	0.00	0	12.8 ± 0.7	52.5 ± 2.1	2.18 ± 0.07
Meal 1 with addition of nitriles	0.03	0.01	0	11.2 ± 0.5	46.7 ± 1.5	2.13 ± 0.05
- " -	0.10	0.04	0	9.1 ± 0.5	43.8 ± 1.2	1.86 ± 0.08
- " -	0.48	0.24	0	-0.7 ± 0.4	18.8 ± 1.0	- -
89 % of meal 1 and 11 % of high-glucosinolate meal without heat treatment	0.00	0.12 *	11	3.8 ± 0.6	30.7 ± 1.5	1.05 ± 0.11
Bronowski meal, no heat treatment	0.00	0.15 *	100	3.1 ± 0.3	26.9 ± 1.0	0.95 ± 0.08

* After autolysis

high-glucosinolate meal resulting in a glucosinolate content of the sample similar to that of a Bronowski meal, was similar to a raw Bronowski meal in feeding value. This suggests that even a low-glucosinolate content together with the presence of native enzyme systems may be responsible of the growth-depressing effects of the raw meal.

A comparison of the results in Table 6 with the results with oxazolidinethiones in Figure 5 suggests that nitriles have much greater growth-depressing effects than oxazolidinethiones. However, adding purified nitriles to a detoxified meal at a level corresponding to the amounts found in autolysed meal, using the analytic method of DAXENBICHLER et al. (1970), did not cause as much growth depression as the autolysed meal (Table 5). But Table 6 shows that the recovery of the added nitriles was low when the samples were analysed according to this method.

Recently, TOOKEY (1973) found a protein in crambe meal that does not hydrolyse epi-progoitrin, but in the presence of thioglucosides promotes the formation of 1-cyano-2-hydroxy-3,4-epithiobutanes. It seems plausible that rapeseed meal contains similar proteins that are not necessary for enzymatic activity but that specify the hydrolytic reaction. Thus, it is possible that the improvement of the nutritional value of low-glucosinolate rapeseed meal by heat treatment may, for the most part, be due to inactivation of the proteins that specify the hydrolysis of glucosinolates in the direction of nitrile production.

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