ANTINUTRITIONAL EFFECTS OF HIGH AND LOW GLUCOSINOLATE RAPESEED MEALS AND PROGOITRIN TOGETHER WITH MYROSINASE IN THE GROWING RAT

R.K. HEANEY[**], M. VERMOREL[*], and G.R. FENWICK[**]

[*]INRA, C.R.Z.V., Theix, 63122 Ceyrat, France.

[**]AFRC Institute of Food Research, Norwich NR4 7UA, U.K.

Rapeseed meal has been shown to have a number of antinutritional effects when fed to laboratory and farm animals (Bunting, 1981 and Bell, 1984). These include a reduction in feed consumption, depression of growth rate and significant increases in the weights of organs such as kidneys, liver and thyroids. Such effects are in part due to the glucosinolates contained in the meal and are often dose-related.

Progoitrin (2-hydroxy-3-butenyl glucosinolate), a major component of winter rapeseed (B. napus), is broken down by the enzyme myrosinase to form 5-vinyloxazolidine-2-thione which is responsible for thyroid dysfunction in rats (Elfving, 1980), and 1-cyano-2-hydroxy-3-butene which can cause enlargement of the liver and kidney (Gould, 1980). Breakdown of progoitrin may also be effected by bacterial enzymes in the digestive tract and thus producing physiological disturbance even in the absence of plant enzymes.

Bille et al. (1983) have shown that over a short feeding period (9 days) growth rate and feed consumption were decreased when progoitrin was included in the diet at 5g/kg. Weights of liver and kidney were increased and the addition of myrosinase caused a significant increase in the weights of liver and thyroid. We have subsequently shown (Vermorel et al., 1986) that the effects of glucosinolates in the diet becomes more apparent after 10 days, when progoitrin added to a control diet caused increases in the weights of livers (+15%), kidneys (+6%) and thyroids (+26%).

The experiment described here studies the effect of myrosinase addition to rat diets containing progoitrin and to diets that included defatted meal from the rapeseed cultivars Jet Neuf and Darmor. The endogenous enzymes of the meals were first inactivated by processing.

Materials and Methods

The two rapeseed meals (cvs Darmor and Jet Neuf) were supplied by CETIOM and analysis showed that they differed in composition only in their glucosinolate content having 37.7 and 100.8 µmoles/g defatted meal respectively. Progoitrin (together with gluconapoleiferin) levels were 27.8 µmoles/g for Darmor and 72.8 µmoles/g for Jet Neuf. Progoitrin was isolated from swede (*B. napus L var napobrassica*) seed (Hanley *et al.*, 1983) and myrosinase was obtained from white mustard (*Sinapis alba*) (Appelqvist and Josefsson, 1967). Diets (balanced in protein, amino acids, trace elements and minerals) were prepared as shown in Table 1.

Table 1. Composition of Diets (g DM/kg DM)

	Diet								
	1	2	3	4	5	6	7	8	9
Herring meal	150	0	0	76	76	150	150	150	150
Darmor meal	0	367	367	0	0	0	0	0	0
Jet Neuf meal	0	0	0	172	172	0	0	0	0
Added progoitrin	0	0	0	0	0	0	3	0	0
Added myrosinase	0	0	1	0	1	0	1	0	0
Methionine	2.5	0	0	1.25	1.25	1.25	2.5	2.5	2.5
Lysine.HCl	0	0.6	0.6	0.3	0.3	0	0	0	0
Starch	769.5	533.4	552.4	672.5	671.5	769.5	769.5	769.5	769.5
Minerals	40	40	40	40	40	40	40	40	40
Vitamins	18	18	18	18	19	18	18	18	18
Corn oil	20	20	20	20	20	20	20	20	20

All diets were fed *ad libitum* except control diets 8 and 9. Diet 8 was dispensed in quantities equal to that consumed by the rats receiving diet 7 (pair feeding). Diet 9 was dispensed in quantities of digestible energy equivalent to that consumed by the rats on the rapeseed diets. The possibility of glucosinolate/myrosinase interaction was avoided by dispensing diets in a dry form using a special feeder. Spillages were recovered and added back to diet refused.

For diets 1-5 Sprague-Dawley rats weighing approximately 78g were used (10 rats per dietary regime). Feeding was for 30 days.

For diets 6-9, groups of 7 rats weighing approximately 70g were fed for 23 days. Animals were progressively introduced to the experimental diets over 3 days with 50% experimental diet and 50% control diet for 2 days, 75% experimental diet for day 3 and 100% experimental diet from day 4 onwards.

Feed consumption was noted daily and cumulated throughout the experiment and animals were weighed 3 x weekly. At the end of the experiment the rats were anaesthetised and sacrificed by intercardiac puncture. Samples of portal and peripheral blood were taken for analysis. Thyroids, livers and kidneys were excised and weighed and after removal of the contents of the digestive tract, small intestine and caecum, empty body weights were recorded.

Table 2. Effects of Glucosinolate level and myrosinase on rat performance (means and standard deviations)

GROUP	1	2	3	4	5
RAPESEED	0	DARMOR		JET NEUF	
GLUCOSINOLATES (mM/kg DM)	0	13.7	13.7	17.3	17.3
MYROSINASE (1 g/kg DM)	0	0	+	0	+
DM INTAKE (g/d)	21.39 ± 1.64	20.01 ± 0.78	19.21 ± 0.80	17.37 ± 1.19	16.50 ± 0.77
D.E* INTAKE (kcal/d)	85.8	69.5	66.9	66.4	63.0
(%)	(100)	(81)	(78)	(77)	(73)
LIVE WEIGHT GAIN (g/d)	7.96 ± 0.59	6.28 ± 0.48	5.90 ± 0.11	5.35 ± 0.49	5.27 ± 0.11
(%)	(100)	(79)	(74)	(67)	(66)
EMPTY BODY GAIN (g/d)	7.80 ± 0.69	6.09 ± 0.29	5.61 ± 0.13	5.21 ± 0.37	5.11 ± 0.11
(%)	(100)	(78)	(72)	(67)	(66)
LIVE WEIGHT GAIN (g/d) during the last 16 days	7.84 ± 1.14	6.82 ± 1.06	5.79 ± 0.40	6.12 ± 0.98	5.04 ± 0.52
(%)	(100)	(87)	(74)	(78)	(64)

*Digestibility energy intake, estimated from the results of previous experiments.

Results

Dry matter intakes and digestible energy intakes were reduced by

both rapeseed diets. Addition of myrosinase to these diets further reduced these values (Table 2).

Addition of myrosinase and progoitrin to the control diet reduced food intake by 13.3% on average during the whole period of the experiment. During the first 16 days the reduction was 9.5% but during the last part of the period intake was down by 25% (Fig. 1).

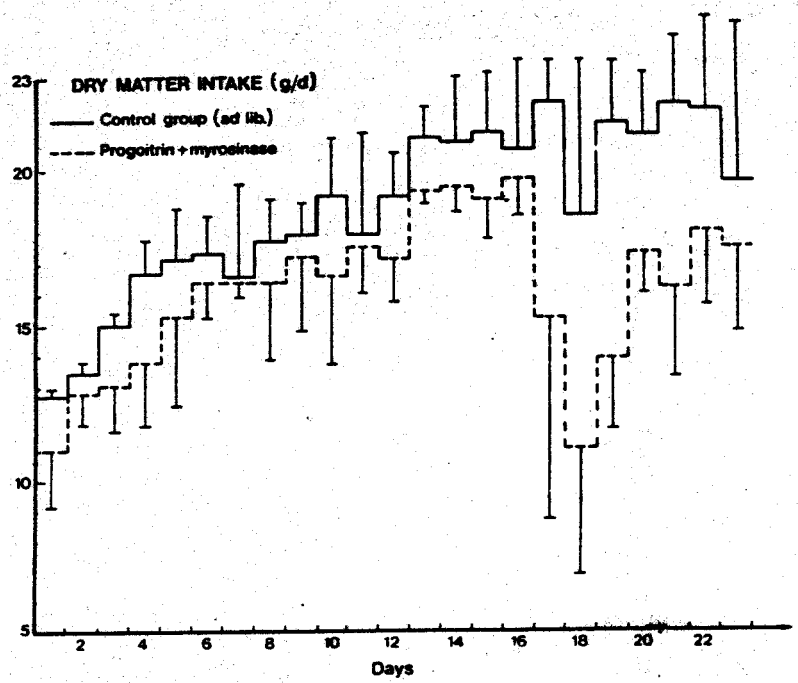

Figure 1. Effect of progoitrin and myrosinase on dry matter intake.

Similarly a reduction in live weight gain of 54% was noted during the final 10 days of feeding the progoitrin plus myrosinase diet compared with a mean value of 23% during the whole period.

The inclusion of Jet Neuf and Darmor meals in diets reduced live weight gains by 21% and 33% respectively and the addition of myrosinase to the diet caused a further reduction of about 5%. The effect of myrosinase addition was more pronounced during the last 16 days with reductions of around 14% compared with diets without myrosinase.

Similarly progoitrin reduced overall weight gain by 24% although during the first 10 days the difference (5%) was not significant. In the final period however, a drop of 54% was noted with some rats gaining no weight or even losing weight.

The inclusion of both rapeseed meals in diets caused an increase in the weights of livers and kidneys (12-14%). Liver weights were further increased by the addition of myrosinase but no such effect was noted for the kidneys (Table 3). Although both rapeseed diets had similar levels of the goitrogen precursor progoitrin, thyroid weights were higher with Darmor (176%) than with Jet Neuf (111%) and the inclusion of myrosinase increased the weights of these organs by a further 80% and 40% respectively. The reasons for this difference are not known.

Animals fed a diet which included progoitrin and myrosinase showed an increase in liver weight but kidneys were unaffected and the increase in the weight of the thyroid (38%) was much less than with rapeseed.

Table 3. Effect of glucosinolate and myrosinase intake on organ weights

Rapeseed study	Organ weights (g/100g L.W) Kidney	Liver	Thyroid
1 Control	0.73 ± 0.05	5.13 ± 0.39	4.46 ± 0.79
(%)	(100)	(100)	(100)
2 Darmor	0.82 ± 0.04	5.84 ± 0.60	12.30 ± 2.36
(%)	(112)	(114)	(276)
3 Darmor + myrosinase	0.79 ± 0.04	6.12 ± 0.43	22.99 ± 4.19
(%)	(108)	(120)	(515)
4 Jet Neuf	0.82 ± 0.05	5.75 ± 0.61	9.40 ± 1.48
(%)	(112)	(112)	(211)
5 Jet Neuf + myrosinase	0.77 ± 0.04	6.14 ± 0.42	13.10 ± 2.75
(%)	(106)	(120)	(294)
Progoitrin study			
6 Control (ad lib)	0.833 ± .002	5.31 ± 0.59	6.01 ± 1.02
(%)	(100)	(129)	(100)
7 Progoitrin + myrosinase	0.846 ± .001	8.37 ± 1.00	8.31 ± 1.15
(%)	(101)	(204)	(138)
8 Control (restricted)	0.842 ± .004	4.11 ± 0.34	6.08 ± 0.54
(%)	(101)	(100)	(101)

The effects of these diets on thyroid hormone levels are less clear. Levels of T_3 were reduced by 50% on feeding Jet Neuf and by a further 50% with inclusion of myrosinase. In contrast, increased levels of T_3 were recorded with Darmor diets, these being reduced by the addition of myrosinase. Plasma T_4 levels were reduced (24%) by both diets and further reduced by the inclusion of myrosinase. Plasma T_3 levels were unaffected by the progoitrin and myrosinase but plasma T_4 levels were down by 56%.

Discussion

The increase in antinutritional effects during the latter part of this experiment clearly indicates the importance of conducting such studies over a sufficiently long period of time. It is only recently that such extended feeding trials have been possible as a result of the development of techniques for isolating relatively large amounts of glucosinolates.

In a previous study (Vermorel et al., 1986) it was shown that dietary progoitrin (3g/kg) increased kidney weight (7%). In this study similar amounts of progoitrin had no effect on kidney weight although liver weights were much higher than for either progoitrin alone or for either rapeseed diet with myrosinase. The exact nature of progoitrin breakdown products may be important in determining the extent of such effects. The addition of myrosinase to progoitrin diets increased thyroid weights by 38% - much less than the effect of either of the rapeseed diets. The two rapeseed diets had similar amounts of progoitrin but in the presence of myrosinase Darmor had a much greater effect on thyroid weights (415%) than Jet Neuf (194%). The reasons for this, and also for the retarded effects on plasma T3 and T4 levels, are not known but may involve subtle differences in the way progoitrin is metabolised. Metabolic studies, in support of such a possibility, are now urgently needed and it is encouraging that one problem - the availability of analytically pure progoitrin has recently been overcome.

The results presented here clearly confirm the role of progoitrin (via its antinutritional and toxic hydrolysis products) in many of the physiological effects which follow the feeding of rapeseed. Reduction of this glucosinolate by selective breeding or by processing the seed

or meal so as to reduce progoitrin levels (and those of its products) below the thresholds necessary for biological activity are thus options which should be considered with a view to the greater exploitation of the nutritional potential of rapeseed meal.

Acknowledgement

Financial support for this work from the Ministry of Agriculture Fisheries & Food (U.K.) and from the Ministére de l'Industrie et de la Recherche (France) is gratefully acknowledged.

References

Appelqvist, L-A; Josefsson, E., 1967. Method for the quantitative determination of isothiocyanates and oxazolidinethiones in digests of seed meals of rape and turnip rape. J. Sci. Food Agric. 1, 510-519.

Bell, J.M., 1984. Nutrients and toxicants in rapeseed meal: a review. J. Anim. Sci. 58, 996-1010.

Bille, N.; Eggum, B.O.; Jacobsen, I.; Olsen, O.; Sørensen, H., 1983. Antinutritional and toxic effects in rats of individual glucosinolates (± myrosinase) added to a standard diet. Effects on protein utilization and organ weights. Z. Tierphysiol. Tierernaehr. Futtenermittelk., 49, 195-210.

Bunting, E.S. (ed), Production and Utilization of Protein In Oilseed Crops, Martinus Nijhoff, The Hague, 1981 and references therein.

Elfving, S., 1980. Studies on the naturally occurring goitrogen 5-vinyl-2-thiooxazolidone. Ann. Clin. Res. 28, 1-47.

Gould, D.H.; Gumbmann, M.R.; Daxenbichler, M.E., 1980. Pathological changes in rats fed the Crambe meal - glucosinolate hydrolytic products 2S-1-cyano-2-hydroxy -3,4-epithiobutanes (erythro and threo) - for 90 days, Food Cosmet. Toxicol., 18, 619-625.

Hanley, A.B.; Heaney, R.K.; Fenwick, G.R., 1983. Improved isolation of glucobrassicin and other glucosinolates. J. Sci. Food Agric. 34, 869-873.

Vermorel, M.; Heaney, R.K.; Fenwick, G.R., 1986. Nutritive value of rapeseed meal: Effects of individual glucosinolates. J. Sci. Food Agric., 37, 1197-1202.