ACCEPTABLE CONCENTRATIONS OF GLUCOSINOLATES IN DOUBLE LOW OILSEED RAPE AND POSSIBILITIES OF FURTHER QUALITY IMPROVEMENTS BY PROCESSING AND PLANT BREEDING

Birthe Bjerg[1], Bjørn O. Eggum[2], Lone Melchior Larsen[1] & Hilmer Sørensen[1]

1) Chemistry Department, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Denmark. 2) The National Institute of Animal Science, Foulum Research Center, Department of Animal Physiology and Biochemistry, DK-8833 Ørum Sønderlyng, Denmark.

ABSTRACT

The quality and nutritive value of rapeseed meal from various double low varieties have been studied in feeding trials with different animals and with application of comprehensive chemical, biochemical and toxicological investigations. The problems previously met with the methods of glucosinolate analysis have been solved. Methods for isolation of appreciable amounts of intact glucosinolates and myrosinases have been developed. The compounds and enzymes have been used in comprehensive studies of antinutritional and toxic effects of glucosinolates and degradation products thereof.

Double low oilseed rape produces plant protein with a well balanced amino acid pattern, and, consequently, a high biological value. Antinutritional and toxic effects of glucosinolates and/or their degradation products reduce, however, the quality of oilseed rape if the concentration of these compounds is too high in diets.

The double low oilseed rape varieties now available have a glucosinolate content in the seeds which is significantly reduced compared to older varieties, and thereby their quality is much improved. Relatively great variations occur, however, in their content and composition of glucosinolates and thereby in their quality. This fact, and previously quite often a lack of reliable determinations of glucosinolates in diets used in feeding experiments have resulted in a lot of the difficulties which obviously exist in establishing what can be considered as acceptable concentrations of glucosinolates in feed to different animals.

Ten different glucosinolates have now been investigated individually in N-balance trials with young rats fed a standard diet containing varying amounts of the intact glucosinolates ± myrosinases and no other specific rapeseed constituents. The results obtained are compared with those from energy and N-balance trials with rats using double low rapeseed meal containing varying amounts of glucosinolates as well as rapeseed subjected to different types of processing. The results are furthermore correlated with those obtained in trials with mink, pigs and calves. It is recommended that such experiments are used as basis for recommendations with respect to the levels and compositions of glucosinolates in diets to animals.

The present knowledge and still vet unsolved problems are presented and briefly discussed.

INTRODUCTION

Rapeseed meal has a relatively high content of protein with a well balanced amino acid composition (Bille et al.,

1983a). It is, however, impossible to obtain an optimal utilization of oilseed rape, if the seeds have a too high content of glucosinolates (Eggum et al., 1985a). The double low oilseed rape varieties now available have a glucosinolate content in their seeds, which is significantly reduced compared to the level in seeds of older varieties. This gives appreciable advantages and possibilities for their utilization in feed to different animals. The composition of individual glucosinolates in double low varieties is different for the different varieties (Møller et al., 1985) and also quite different from that of the older varieties, including single low varieties. The quality of rapeseed meal from different rape varieties varies with their content of glucosinolates and/ /or degradation products thereof.

Knowledge of types and concentrations of glucosinolates in double low oilseed rape as well as their properties is of utmost importance. These informations are required for determination of which level of glucosinolates in rapeseed there can be considered as acceptable for utilization of rapeseed without problems. Use of reliable methods of analysis for determination of all types of glucosinolates are required (Bjerg and Sørensen, 1987a). Otherwise it is absurd to discuss these problems, and feeding trials with rapeseed meal where such methods have not been used to control the glucosinolate content and composition in the diets are of little value. Information on the possible glucosinolate degradation during extraction of oil and other applied processing procedures need also to be evaluated (Bille et al., 1983a; Eggum et al., 1985b; Bjerg et al., 1986a). Our recent research has comprised investigation of these problems. The studies have included: (a) Investigations based on pure individual glucosinolates, (b) myrosinases (β-thioglucoside glucohydrolase, EC 3.2.3.1), (c) rapeseed and rapeseed products with and without processing, (d) evaluation of the various effects and properties of the compounds and products in N-balance trials with rats, (e) feeding trials with different animals. Results and conclusions from these investigatons are briefly presented and discussed in this paper.

MATERIALS AND METHODS

Details and comprehensive descriptions of the applied methods are presented elsewhere:

(a) Isolation of glucosinolates (Bille et al., 1983b; Bjerg and Sørensen, 1987b)
(b) Methods of analysis for glucosinolates (Bjerg and Sørensen, 1987a; Bjerg et al., 1987a)
(c) Various chemical analysis, processing procedures and N-balance trials with rats (Bille et al., 1983a; Bjerg et al., 1986a)
(d) Isolation and investigation of myrosinases (Bille et al., 1983b; Buchwaldt et al., 1986)
(e) Isolation and investigation of aromatic choline esters and aromatic choline esterase (Clausen et al., 1985a; ibid 1985b; Clausen et al., 1986)
(f) N-balance trials with rats, antinutritional and toxic effects of individual glucosinolates ± myrosinases (Bille et al., 1983b; Bjerg et al., 1986b)
(g) Trials with mink (Henriksen et al., 1987)
(h) Trials with pigs and sows (Eggum et al., 1985c; Danielsen et al., 1987)
(i) Trials with young bulls (Andersen and Sørensen, 1985).

RESULTS AND DISCUSSION

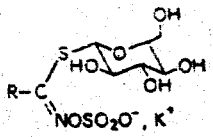
Table 1 shows some selected results obtained with meal from seeds of Brassica napus and B. campestris cultivars. Further details, including information on the N-balance trials with growing rats used for evaluation of the protein quality, antinutritional or toxic effects are presented in previous publications (Bille et al., 1983a; Bjerg et al., 1986a). With a sufficient low level of glucosinolates, it is possible to obtain high biological values in accordance with the well balanced amino acid composition of rapeseed meal.

High content of glucosinolates in the diets reduces the BV values, but the reduction does not follow the total glucosinolate content. It has also been found (Clausen et al., 1986) that the aromatic choline esters have only little effect on the protein utilization, but these compounds are of interest for other reasons in relation to the rapeseed quality (Eggum et al., 1985a).

Table 1. Chemical composition, true protein digestibility (TD), biological value (BV), net protein utilization (NPU) of B. napus and B. campestris meal samples.

	Control Starch,Met, Casein	Meal from Brassica seeds				
		B. napus		B. campestris		
		1	2	3	4	5
Protein (N x 6.25)		40.5	39.6	33.2	40.2	38.3
Stoldt fat %		4.4	4.1	14.9	5.2	17.4
RHC %		12.9	10.4	14.4	13.4	4.8
Crude fibre %		14.4	14.4	14.2	11.7	15.1
Ash		8.3	7.8	8.8	8.8	10.0
Amino acids (g/16g N):						
Lysine		5.84	5.72	7.06	5.87	7.21
Threonine		4.64	4.51	4.78	4.60	4.75
Tryptophan		1.29	-	1.27	-	1.31
Methionine + Cysteine		4.40	4.04	5.23	4.33	5.23
Glucosinolates (µmole/g):						
Glucoraphanin		0.1	0.2	-	0.2	-
Glucoalyssin		0.2	0.3	-	0.2	-
Progoitrin		5.3	4.7	-	4.1	-
Napoleiferin		0.2	0.7	-	0.9	-
Gluconapin		2.6	2.0	54.4	3.0	16.1
Glucobrassicanapin		0.7	1.1	4.8	2.0	2.4
4-Hydroxyglucobrassicin		1.2	0.2	-	0.4	-
Glucobrassicin		0.1	0.7	0.7	0.4	0.3
Other		0.9	0.6	-	2.2	-
Total		11.3	10.5	59.9	13.4	18.8
Aromatic choline esters (µmole/g):						
Sinapine		19.2	25.2	15.2	18.7	7.2
Other		7.2	5.8	6.8	6.3	2.4
TD %	99.8	84.8	84.9	87.1	85.0	85.9
BV %	91.2	97.1	86.0	53.5	90.3	86.2
NPU %	90.6	73.8	73.0	46.6	76.8	74.1

To obtain information on acceptable dietary glucosinolate levels and toxic effects of the individual glucosinolates and/or their degradation products, several glucosinolates ± myrosinases have been investigated (Bille et al., 1983b; Bjerg et al., 1986b). Structures and names of the ten glucosinolates investigated in balance trials with young growing rats are shown in Figure 1, and results from the trials are shown in Tables 2 and 4.



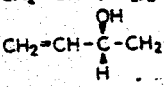
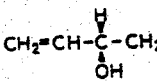
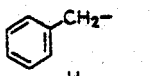
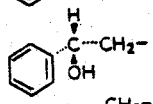
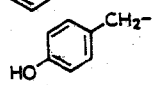
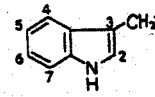
	R-group	Semisystematic names	Trivial names
1	$CH_2=CH-CH_2-$	Allylglucosinolate	Sinigrin
2	$CH_2=CH-(CH_2)_2-$	But-3-enylglucosinolate	Gluconapin
3		(2R)-2-Hydroxybut-3-enylglucosinolate	Progoitrin
4		(2S)-2-Hydroxybut-3-enylglucosinolate	Epiprogoitrin
5	$CH_3-SO-(CH_2)_3-$	3-Methylsulfinylpropylglucosinolate	Glucoiberin
6	$CH_3-SO-(CH_2)_4-$	4-Methylsulfinylbutylglucosinolate	Glucoraphanin
7	$CH_3-SO_2-(CH_2)_3-$	3-Methylsulfonylpropylglucosinolate	Glucocheirolin
8		Benzylglucosinolate	Glucotropaeolin
9		(2S)-2-Hydroxy-2-phenyl-ethylglucosinolate	Glucobarbarin
10		p-Hydroxybenzylglucosinolate	Sinalbin
11		3-Indolylmethylglucosinolate	Glucobrassicin

Figure 1. Structures and names of glucosinolates investigated individually (except 11) in balance trials with young rats ± myrosinases added.

Table 2. Effects of individual glucosinolates ± myrosinases on true protein digestibility (TD), biological value (BV) and net protein utilization (NPU).

Glucosinolate No. (Fig.1)	Glucosinolate concentration											
	1. nivesu 0.5 µmole/g			2. nivesu 2.5 µmole/g			3. nivesu 12.5 µmole/g			4. nivesu 2. + myrosinases		
	TD	BV	NPU	TD	BV	NPU	TD	BV	NPU	TD	BV	NPU
1	100	89.7	89.6	100	87.4	87.7	99.2	75.9	75.3	99.7	81.9	81.7
2	97.8	93.9	92.6	98.9	92.1	91.0	96.2	89.6	86.2	97.4	90.8	88.5
3	99.9	89.9	89.7	99.5	88.2	87.7	99.6	77.9	77.6	97.5	83.7	81.6
4	98.6	89.8	88.6	97.6	88.4	86.3	97.1	84.2	81.7	97.5	87.3	85.1
5	98.9	93.4	92.3	99.0	89.3	88.3	96.4	83.3	80.4	98.0	87.3	85.6
6	98.0	91.5	89.7	98.9	90.8	89.7	–	–	–	98.9	90.1	89.1
7	98.0	91.1	89.3	98.3	91.2	89.7	96.9	82.2	79.5	98.6	84.6	83.4
8	98.3	89.6	88.1	98.3	90.3	88.8	97.8	84.9	83.0	98.5	86.4	85.0
9	99.2	89.9	89.1	99.1	90.2	89.4	99.5	90.0	89.6	99.1	90.3	89.5
10	100	88.2	88.2	99.6	84.2	83.8	99.0	81.7	80.9	99.5	84.3	83.8

Table 3. Glucosinolate level in seed and rapeseed meal corresponding to the concentrations used in the diets.

	1. niveau	2. niveau	3. niveau	4. niveau
Diet: µmole/g DM	0.5	2.5	12.5	concentration as in 2. niveau but with added myrosinases
Feed with 20% rapeseed meal: µmole/g rapeseed meal	2.5	12.5	62.5	
Seed before oil extraction: (ca. µmole/g seed), depend on oil and water content	1-2	6-8	30-40	

Table 4. Effects from the glucosinolates; *, weak (*) or (∇), reduced weight ∇.

Glucosinolate No. (Fig. 1)	Weight gain	BV	TD	Liver	Kidneys	Testicles	Thyroid
1	*	*	-	-	*	-	(∇)
2	-	*	(*)	-	-	-	∇
3	*	*	-	-	*	-	*
4	(*)	*	-	*	-	-	(∇)
5	(*)	*	(*)	-	-	-	(∇)
6	-	(*)	-	-	*	-	-
7	(*)	*	(*)	-	-	-	-
8	(*)	*	-	-	*	-	-
9	(*)	-	-	*	*	*	(*)
10	(*)	*	-	-	-	-	*

The different glucosinolates or degradation products thereof have various effects on the animals. The effects are seen at varying levels in the diets. Antinutritional and/or toxic effects can be caused by intact glucosinolates, but especially in combination with myrosinases. Inactivation of myrosinases can reduce but not solve the problems caused by glucosinolates. It is found that the results and conclusions obtained with young rats in balance trials to study the effects from pure compounds and from rapeseed with and without different types of processing are in agreement with results obtained vith young bulls (Andersen and Sørensen, 1985). It is also found that growing - finishing pigs have corresponding requirements to the rapeseed quality (Eggum et al., 1985c), and the experiments with mink (Henriksen et al., 1987) as well as the long-term sow experiments (Danielsen et al., 1987) indicate that a slightly lower glucosinolate level is recommend-

able to avoid antinutritional and toxic problems. This means a level around 1. - and 2. niveau (Table 3) in diets to pigs, depending on the type of glucosinolates, when the myrosinases are inactivated and the glucosinolates are intact after oil extraction and processing. For the animals included in the experiments performed (Eggum et al., 1985b) most of the glucosinolates and aromatic choline esters (Clausen et al., 1986), but not the total amount, disappear in the first part of the small intestine. We need, however, much more information about the metabolism of glucosinolates in the animals, other problems also call for attention (Eggum et al., 1985a), and it is recommendable to use the best possible double low varieties.

REFERENCES

1. Bille, N., Eggum, B.O., Jacobsen, I., Olsen, O. and Sørensen, H. (1983a): The effects of processing in antinutritional rape constituents and the nutritive value of double low rapeseed meal. Zeitschr. Tierernährung u. Futtermittelkd. 40, 148-163.
2. Eggum, B.O., Larsen, L.M., Poulsen, M.H. and Sørensen, H. (1985a): Conclusions and recommandations. In: Advances in the Production and Utilization of Cruciferous Crops. (Ed. Sørensen, H.) Martinus Nijhoff/Dr. W. Junk Publ., Dordrecht/Boston/Lancaster. pp. 304-311.
3. Møller, P., Olsen, O., Plöger, A., Rasmussen, K.W. and Sørensen, H. (1985): Quantitative analysis of individual glucosinolates in double low rapeseed by HPLC of intact glucosinolates. ibid. pp. 111-126.
4. Bjerg, B. and Sørensen, H. (1987a): Quantitative analysis of glucosinolates in oilseed rape based on HPLC of desulfoglucosinolates and HPLC of intact glucosinolates. In: Proceedings from CEC Workshop, Glucosinolates in Rapeseeds, Analytical Aspects, October 1986 in Gembloux, Belgium. in press.
5. Eggum, B.O., Olsen, O. and Sørensen, H. (1985b): Effects of glucosinolates on the nutritive value of rapeseed. In: Advances in the Production and Utilization of Cruciferous Crops. (Ed. Sørensen, H.) Martinus Nijhoff/Dr. W. Junk Publ., Dordrecht/Boston/Lancaster. pp. 50-60.
6. Bjerg, B., Eggum, B.O., Jacobsen, I., Larsen, L.M., Rahman, M.H. and Sørensen, H. (1986a): Ernæringsmæssig kvalitet af mørkskallet dobbeltlav vårraps (Topas) og af gulskallet raps i relation til glucosinolatindhold, tannin og procesbehandling. Statens Husdyrbrugsforsøg, Meddelelse nr. 634, 1-4.
7. Bille, N., Eggum, B.O., Jacobsen, I., Olsen, O. and Sørensen, H. (1983b): Antinutritional and toxic effects in rats of individual glucosinolates (± myrosinases) added to a standard diet. 1. The effects on protein utilization and organ weight. Zeitschr. Tierphysiol., Tierernährung u. Futtermittelkd. 40.148-163.
8. Bjerg, B. and Sørensen, H. (1987b): Isolation of intact glucosinolates by column chromatography and determination of their purity. In:

Proceedings from CEC Workshop, Glucosinolates in Rapeseeds, Analytical Aspects, October 1986 in Gembloux, Belgium. in press.

9. Bjerg, B., Eggum, B.O., Larsen, L.M. and Sørensen, H. (1987a): Reliability of analytical methods for quantitative determination of individual glucosinolates and total glucosinolate content in double low oilseed rape. Contribution to: 7th International Rapeseed Congress, Poznan, Poland. in press.
10. Buchwaldt, L., Larsen, L.M., Plöger, A. and Sørensen, H. (1986): FPLC isolation and characterization of plant myrosinase, β-thioglucoside glucohydrolase, isoenzymes.
J. Chromatogr. 363(1), 71-80.
11. Clausen, S., Larsen, L.M., Plöger, A. and Sørensen, H. (1985a): Aromatic choline esters in rape seed. In: Advances in the Production and Utilization of Cruciferous Crops. (Ed. Søresnen, H.) Martinus Nijhoff /Dr. W. Junk Publ., Dordrecht/Boston/Lancaster. pp. 61.72.
12. Clausen, S., Larsen, L.M., Plöger, A. and Sørensen, H. (1985b): Aromatic choline esterase and catabolism of aromatic choline esters.
ibid. pp. 138-147.
13. Clausen, S., Eggum, B.O., Jacobsen, I., Larsen, L.M., Plöger, A. and Sørensen, H. (1986): Individuelle aromatiske cholinestere og deres bidrag til tannin: Kvalitets- og ernæringsmæssige problemer ved rapsfrøprodukter.
Statens Husdyrbrugsforsøg, Meddelelse nr. 609, 1-4.
14. Bjerg, B., Eggum, B.O., Jacobsen, I., Jensen, J., Larsen, L.M. and Sørensen, H. (1986b): Ernæringsmæssige og toksiske effekter af seks individuelle glucosinolater iblandet en glucosinolatfri diæt og fodret til rotter.
ibid. Meddelelse nr. 619, 1-4.
15. Henriksen, P., Hillemann, G., Mortensen, K. and Sørensen, H. (1987): Requirements to the quality of oilseed rape which can be used without problems in diets to mink (Mustela vison Screb.) Contribution to: 7th International Rapeseed Congress, Poznan, Poland. in press.
16. Eggum, B.O., Just, A. and Sørensen H. (1985c): Double low rapeseed meal in diets to growing - finishing pigs. In: Advances in the Production and Utilization of Cruciferous Crops. (Ed. Sørensen, H.) Martinus Nijhoff/Dr. W. Junk Publ., Dordrecht/ Boston/Lancaster. pp. 167-176.
17. Danielsen, V., Eggum, B.O., Rasmussen, K.W. and Sørensen, H. (1987): Long-term studies of requirements to the quality of rapeseed meal from double low varieties used in sow diets. Contribution to: 7th International Rapeseed Congress, Poznan, Poland. in press.
18. Andersen, H.R. and Sørensen, H. (1985): Double low rapeseed meal in diets to young bulls. In: Advances in the Production and Utilization of Cruciferous Crops. (Ed. Sørensen, H.) Martinus Nijhoff/Dr. W.Junk Publ., Dordrecht/Boston/Lancaster. pp.208-217.