

Counteracting the negative effects of rapeseed and rapeseed press cake in pig diets*

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Rapeseed and rapeseed press cake were tested in four long-term experiments with a total of ninety-eight pigs. Rapeseed contained 20 and rapeseed press cake 19 mmol glucosinolates/kg DM. The proportion of the tested rapeseed products in feed amounted to 0 (control), 50, 100 and 150 g/kg diet. Moist-heat-treated rapeseed and rapeseed press cake with an extremely low glucosinolate content were also given at 150 g/kg diet. Each dietary rapeseed product level was given with 125 or 250 µg supplementary I/kg diet. Reduced feed intake and growth retardation were found in groups receiving 150 g rapeseed products/kg diet; in the case of rapeseed the impairments were significant. Rapeseed products ≥ 100 g/kg diet increased the thyroid weight and decreased the serum thyroxine (T₄) concentration. Higher I dosage brought the serum T₄ concentration to the level of the control group and retarded thyroid enlargement. Intake of rapeseed products lowered the I content of the thyroid; however, there was no significant difference between groups given 0.9 mmol glucosinolates/kg diet and those receiving three times as much. Degrading glucosinolates by moisture and heat prevented feed intake depression and growth retardation. In the case of treated rapeseed the decreased serum T₄ concentration and increased thyroid weight persisted, indicating formation of some antithyroid compounds due to myrosinase (EC 3.2.3.1) activation. A maximal glucosinolate content of 2 mmol/kg diet and additional I application are a prerequisite for using rapeseed products in pig feeding.

Rapeseed: Iodine: Thyroid hormones: Pigs

In Germany, rapeseed (*Brassica napus* var. *oleifera*) products are the most important home-grown protein feeds, representing 7% of the 20 million tonnes annual compound feed production. The processed residue of solvent-extracted rapeseed meal from oil mills dominates. Ground rapeseed acts as a temporary energy feed in case of low rapeseed prices and rapeseed press cake, a newer feed processed in smaller screw presses in agricultural plants (Lüdke & Schöne, 1994) combines advantageous contents of protein (300–350 g/kg) and fat (100–200 g/kg).

The success of rapeseed feeds is due to the creation of double zero varieties (<20 g erucic acid/kg, <25 mmol glucosinolates/kg, based on 910 g/kg DM). Glucosinolates may impair feed intake, growth and thyroid function in farm animals, particularly in pigs and poultry. A large number of experiments with rapeseed meal or ground rapeseed from high-

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glucosinolate cultivars have demonstrated significant feed intake depression, growth retardation, enlargement of thyroid or liver and of the corresponding parenchyma cells respectively, and a decreased serum thyroxine (T_4) concentration (McKinnon & Bowland, 1979; Rowan & Lawrence, 1986; Schöne *et al.* 1991a,b; Spiegel *et al.* 1993). In experiments with rapeseed from newly bred low-glucosinolate cultivars the animal response seems to be low (Rundgren, 1983; Bell, 1984; Bourdon & Aumaître, 1990; Schöne *et al.* 1991a). However, a safe level of use for rapeseed products has still to be defined with dose-response experiments (Campbell & McDonald, 1991).

Glucosinolates are found with the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1). In the intact plant the enzyme is located in cellular compartments which are separated from the glucosinolates (Maheshwari *et al.* 1981). Crushing of seed and soaking in water (Schöne *et al.* 1994b) brings the myrosinase into contact with glucosinolates, resulting in almost complete disappearance of glucosinolates, isothiocyanates and further unstable glucosinolate degradation products. Effects of moist heat treatment and myrosinase activation in rapeseed products on animals seem to be unclear. In experiments of Münchmeyer *et al.* (1974) pigs avoided myrosinase-treated high-glucosinolate rapeseed meal diets. More recently in low-glucosinolate rapeseed products the glucosinolate degradation by endogenous myrosinase activation increased diet acceptance by rats (Dietz *et al.* 1991) or pigs (Schöne *et al.* 1994b).

Glucosinolates and glucosinolate degradation products impair the I status or increase I requirement. In East Germany pig diets containing high-glucosinolate rapeseed meal were not supplemented with I until 1986, and pigs suffered from hypothyroidism, i.e. cretinism, changed body proportions and shortened limbs, myxoedema and pronounced goitre (Lüdke & Schöne, 1988). The National Research Council (1988) recommends 140 µg (supplementary) I/kg feed for growing pigs. The question remains whether this amount of I meets requirements in the case of rapeseed feeds.

The objective of the present experiments was to evaluate the pig response to increasing dietary glucosinolate content combined with different concentrations of I. The glucosinolate content of diets was varied either by changing the proportion of ground rapeseed or rapeseed press cake, or by moist heat treatment. Animal response criteria were the feed intake and live-weight gain, the serum concentrations of thyroid hormone and thiocyanate (SCN^-) and the weight and I content of the thyroid.

MATERIALS AND METHODS

Rapeseed and rapeseed press cake tested

Rapeseed, winter cultivar 'Madora', breeder Saatzucht Hans Lembke GmbH Malchow, Poel, Germany, contained on a DM basis 224 g crude protein/kg, 444 g ether extract/kg and 89 g crude fibre/kg.

Rapeseed press cake, winter cultivar 'Falcon', breeder Norddeutsche Pflanzenzucht Hans Georg Lembke KG Hohenlieth, Germany, was pressed in a screw press (Reinartz Neuss, Germany, Type AP 10/06). Feed contained 321 g crude protein, 180 g ether extract and 102 g crude fibre/kg DM. According to digestibility experiments this rapeseed press cake provided 14.1 MJ metabolizable energy (ME)/kg DM, similar to the energy content of barley (Lüdke & Schöne, 1994).

Rapeseed and rapeseed press cake, both untreated, contained 20 and 18.5 mmol glucosinolates/kg DM (Table 1) respectively. Moist heat treatment has already been described in a previous paper (Schöne *et al.* 1994b). Crushed rapeseed and rapeseed press cake were soaked in tap water (1 litre/kg) for 2 h. Subsequently, the mash was dried at 60°

Table 1. *Glucosinolate concentrations in rapeseed (RS) and rapeseed press cake (RPC) (mmol/kg DM), untreated or moist-heat treated, tested in pig experiments*

Experiment...	1	2	3	4
	RS* untreated	RPC† untreated	RS* soaked‡ and dried	RPC† soaked‡ and dried
Gluconapin	4.0	3.1	0.4	ND
Glucobrassicinapin	1.3	0.6	0.1	ND
Progoitrin	10.8	7.3	1.2	0.1
Pronapoleiferin	0.2	0.2	ND	ND
4-Hydroxy-glucobrassicin	2.9	4.7	0.2	0.2
Glucobrassicin	0.1	0.3	ND	ND
Neoglucobrassicin	0.1	0.1	ND	ND
Others§	0.5	2.2	0.2	ND
Total	19.9	18.5	2.1	0.3

ND, not detectable (<0.1 mmol/kg DM).

* Winter cultivar 'Madora' (one batch).

† Winter cultivar 'Falcon' (one batch).

‡ Crushed RS or RPC (1 kg) was soaked with 1 litre water in a feed mixer and the mash dried to constant weight at 60°

§ Glucoraphanin, glucoallysin, gluconasturtiin and undetectable glucosinolates.

Treatment drastically decreased the glucosinolate content. Glucosinolate degradation products could not be detected in treated rapeseed and rapeseed press cake.

Animals and diets

Four experiments with a total of ninety-eight crossbred pigs (Piétrain × German Landrace × Large White) were carried out. In Expts 1 and 2 dietary proportions of rapeseed products up to 150 g/kg were compared with control diets without rapeseed products. In Expts 3 and 4 150 g moist-heat-treated rapeseed products/kg diet were tested against untreated material. Rapeseed and rapeseed press cake used were the same as in Expts 1 and 2. Ultimately the groups fed with 150 g (untreated) rapeseed products/kg diet from Expts 1 and 2 served as controls for groups receiving this content of rapeseed products treated in Expts 3 and 4. After weaning at 5 weeks of age, the piglets were selected from a total of forty litters and transferred from the pig farm (Gleißberg agrar Golmsdorf, Germany) to the animal nutrition research unit in Remderoda, Germany. For 6 weeks before the experiments, the piglets received a grain–soyabean meal–whey diet (no fishmeal) containing 195 g crude protein, 13 MJ ME, 11 g lysine and 150 µg I/kg feed.

At the start of the experiments the pigs were randomized with respect to body weight, sex and litter. All pigs were held under the same conditions in individual pens (temperature 17–22°, relative air humidity 50–80%), with free access to feed and water. Design and procedures of these animal experiments were approved by the Official Commission for Animal Experimentation (permission of Thuringian Ministry of Health and Welfare dated 18 March 1991).

Expt 1

Forty pigs (twenty female and twenty male castrated) with an initial body weight of 31.5 (SE 0.36) kg were randomly allocated to ten homogeneous groups each consisting of two female and two male castrated pigs from different litters (Table 2; groups 1–10). For a period of 105 d eight animals each received diets without oilseed (groups 1 and 2), with

Table 2. *Composition of rapeseed diets tested in Expt 1* and Expt 3† (g/kg diet)*

Group‡...	1+2	3+4	5+6	7+8	9+10	11+12
Ingredients						
Soyabean meal, solvent-extracted (430 g crude protein/kg)	140	—	128	116	102	102
Soyabean, toasted	—	200	—	—	—	—
Rapeseed, untreated	—	—	50	100	150	—
Rapeseed, treated§	—	—	—	—	—	150
Barley	415	385	396	377	359	359
Wheat	415	385	396	377	359	359
Mineral and vitamin premix	30	30	30	30	30	30
Composition						
DM	874	872	870	871	876	879
Crude protein	174	178	178	180	174	179
Metabolizable energy¶ (MJ/kg)	12.7	13.3	13.0	13.2	13.5	13.6

* Groups 1–10.

† Groups 9–12.

‡ Groups 1, 3, 5, 7, 9 and 11 received 125 µg supplementary I/kg diet; groups 2, 4, 6, 8, 10 and 12 received 250 µg supplementary I/kg diet.

§ Treatment described in Table 1.

|| Supplementation per kg diet: lysine 1.5 g, Ca 4 g, P 1.3 g, Na 1.1 g, Cu 20 mg, Zn 60 mg, Se 0.15 mg, retinol equivalents (as coated retinyl ester preparation) 1.35 mg, cholecalciferol 14 µg, α-tocopherol (as α-tocopheryl acetate preparation) 15 mg, cobalamin 15 µg.

¶ Calculated according to the Deutsche Landwirtschaftsgesellschaft (1991) feed table and Lüdke & Schöne (1994).

200 g toasted soyabeans/kg (groups 3 and 4), or with rapeseed, 50 g/kg (groups 5 and 6), 100 g/kg (groups 7 and 8) or 150 g/kg (groups 9 and 10). The odd-numbered groups 1, 3, 5, 7 and 9 were given the recommended I dose (125 µg supplementary I/kg diet), whereas the even-numbered groups 2, 4, 6, 8 and 10 were given double the recommended dose (250 µg supplementary I/kg diet). In basal diets levels of less than 60 µg I/kg were detected. I was provided by KI bound to casein. This preparation was found to be highly stable (Groppel, 1986). In the diets with 125 µg supplementary I/kg total I concentrations from 90 µg/kg (group 1) to 130 µg/kg diet (group 9) were detected. In the diets with 250 µg supplementary I/kg, the determined total I concentrations ranged from 180 µg/kg diet (group 10) to 230 µg/kg diet (group 2).

Expt 2

In the 111 d feeding experiment eight homogeneous groups of five pigs each (each group consisting of three female and two male castrated pigs from different litters) with a mean initial body weight of 22.5 (SE 0.25) kg received diets with an increasing rapeseed press cake content (Table 3, groups 1–8). The same amounts of ME as well as crude protein and lysine were calculated for all groups (12.4 MJ/kg, 154 g/kg and 8.5 g/kg diet respectively). Pigs of odd-numbered groups 1, 3, 5 and 7 received 125 µg supplementary I/kg diet; pigs of even-numbered groups 2, 4, 6 and 8 were given 250 µg supplementary I/kg diet. The I content of basal diets was the same as in Expt 1. In the low I dosage groups (125 µg/kg diet) the amount of I recovered ranged from 140 (group 7) to 170 µg/kg diet (group 5), and in the high I dosage groups (250 diet) from 260 (group 8) to 330 µg/kg diet (group 4).

Table 3. *Composition of rapeseed press cake diets tested in Expt 2* and Expt 4† (g/kg diet)‡*

Group§...	1+2	3+4	5+6	7+8	9+10
Ingredients					
Soyabean meal (430 g crude protein/kg)	150	122	95	66	66
Rapeseed oil	5	—	—	—	—
Rapeseed cake, untreated	—	50	100	150	—
Rapeseed cake, treated	—	—	—	—	150
Barley	815	798	775	754	754
Mineral and vitamin premix¶	30	30	30	30	30
Composition					
DM	860	858	861	861	865
Crude protein	167	166	170	169	172

* Groups 1–8.

† Groups 7–10.

‡ Metabolizable energy was calculated as 12.4 MJ/kg diet according to the Deutsche Landwirtschaftsgesellschaft (1991) feed table and Lüdke & Schöne (1994).

§ Groups 1, 3, 5, 7 and 9 received 125 µg supplementary I/kg diet; groups 2, 4, 6, 8 and 10 received 250 µg supplementary I/kg diet.

|| Treatment described in Table 1.

¶ Supplementation per kg diet: lysine 1.5 g, Ca 4 g, P 1.3 g, Na 1.1 g, Cu 20 mg, Zn 60 mg, Se 0.15 mg, retinol equivalents (as coated retinyl ester preparation) 1.35 mg, cholecalciferol 14 µg, α -tocopherol (as α -tocopheryl acetate preparation) 15 mg, cobalamin 15 µg.

Expts 3 and 4

The experiments were carried out with a total of thirty-six pigs of which eighteen had already been used in Expts 1 and 2. Moist-heat-treated rapeseed and rapeseed press cake were each fed at a level of 150 g/kg diet (Table 2, groups 11 and 12; Table 3, groups 9 and 10), corresponding to the groups receiving the largest quantity of untreated rapeseed (Table 2, groups 9 and 10) and rapeseed press cake (Table 3, groups 7 and 8) in their diets. The DM and crude nutrient contents of treated rapeseed or rapeseed press cake were similar to those of untreated feed. Duration of experiments, genetics, sex of animals, I dosages and I recovery were similar to those in Expts 1 and 2 (Table 2, Table 3).

Investigation criteria, samples and analyses

Glucosinolates of rapeseed (Expt 1, Expt 3) and rapeseed press cake (Expt 2, Expt 4) were detected by HPLC with sinigrin as internal standard (European Community, 1990). Aglucones were determined by temperature programmed GC and mass spectrometry with phenylisothiocyanate as internal standard (Lange *et al.* 1986). I content of feed ingredients and diets was measured by the method of Sandell & Kolthoff (1937) with HCl–HClO₄ ashing and further modifications introduced by Gstrein *et al.* (1979).

The pigs were weighed once fortnightly and feed intake was recorded. The thyroid was removed at slaughter. The organ was weighed and frozen in a polyethylene bag at –20°. Blood was sampled from the anterior vena cava on day 56, and on the penultimate day in Expts 1 and 3 after a 12 h fasting period. The same procedure as for day 56 was adopted in Expts 2 and 4, however, at the end of the experiments blood was sampled in the slaughterhouse after a 20 h fasting period. Blood samples were transferred to glass tubes, centrifuged after 2 h for 15 min at 1600 g and the serum frozen at –20° until analysed.

The T_4 and T_3 concentrations in serum were measured by radioimmunoassay (RIA Kit Hennig, Berlin, Germany), and the SCN^- concentration by HPLC (Rudolph 1993).

Thyroidal I was measured according to the method of Tiran *et al.* (1991). Lyophilized ground thyroid 4–6 mg (Laboratory scale RC 210 P, Sartorius, Göttingen, Germany) was ashed with $NaClO_3$ (Merck, Darmstadt, Germany, no. 6423) and $HClO_4$ (Merck no. 519) dissolved in distilled deionized water by an Al thermoblock (100°, 25 min). Arsenite reduction solution ($NaAsO_2$, Merck no. 6287) was added to samples and to standards (ashing solution blank or 5, 10, 20 and 30 ng I/ml as KIO_3 , Merck no. 5051). Application of $Ce(SO_4)_2$ (Merck no. 9092) induces the so-called Sandell–Kolthoff reaction: As (III) oxidizing to As (V) reduces Ce (IV) to Ce (III). I catalyses the decolorization of Ce (IV), which is detected photometrically at 405 nm ('Specol 11', Carl Zeiss, Jena, Germany).

Statistical methods

All data were analysed with the Statistical Analysis Systems software package (SAS 6.11, SAS Institute, Heidelberg, Germany). The results are given as means with their (pooled) standard error. Data were analysed by two-way ANOVA with the variables dietary glucosinolate content, I dosage and interaction. Group means were compared using the Newman–Keuls test (Steel & Torrie, 1980).

RESULTS

Rapeseed (Expt 1)

Pigs receiving the highest level of rapeseed (150 g/kg diet) showed the lowest feed intake (Table 4). According to the Newman–Keuls test, however, all differences between group means were below the minimum significant difference of 0.35 kg daily feed intake. Weight gain followed energy intake. In the controls receiving solvent-extracted soyabean the lower weight gain resulted from the lower ME content of this oilseed-free diet (Table 2); in pigs receiving the highest rapeseed level ($P < 0.05$ only with 250 μ g supplementary I/kg diet) it was caused by the decreased feed intake (Table 4).

The feed:gain ratio of pigs receiving control diets containing solvent-extracted soyabean meal was increased ($P < 0.05$ with 125 μ g supplementary I/kg diet) due to lower ME (Table 2).

There was a drastic increase in serum SCN^- concentration due to rapeseed in the diet. Comparing the SCN^- response to 50 g rapeseed/kg diet and to 150 g rapeseed/kg diet, the threefold intake only doubled SCN^- serum concentration. This indicates a lower glucosinolate degradation or better SCN^- elimination with higher quantities of glucosinolates.

At each blood sampling, the I dosage was found to have affected the serum T_4 concentration significantly. In the twenty animals receiving 125 μ g supplementary I/kg diet, serum T_4 levels of 43 (SE 1.8) nmol/l and 48 (SE 2.1) nmol/l were found at day 56 and day 105 respectively, whereas in those receiving 250 μ g supplementary I/kg diet serum T_4 levels reached 51 (SE 1.8) nmol/l and 57 (SE 1.9) nmol/l respectively. The decrease of serum T_4 concentration occurred primarily with diets containing at least 100 g rapeseed/kg. At day 105 the difference in serum T_4 concentration between the low and high I dosage groups was highly significant in the animals receiving at least 100 g rapeseed/kg diet.

The serum T_3 concentration responded to dietary I and glucosinolates significantly only at day 56. The highest serum T_3 concentration was found in pigs with 100 or 150 g rapeseed/kg diet with low I dosage.

Table 4. Expt 1. Responses of pigs (initial body weight 31.5 kg) to graded levels of rapeseed in the diet over 105 d*
(Mean values for four pigs per group, with their pooled standard error)

Ground rapeseed, untreated (g/kg diet)...	0		50		100		150		SEM
	125	250	125	250	125	250	125	250	
Glucosinolates content (nmol/kg diet)...	<0.1	<0.1	0.9	1.8	2.7				
Iodine dosage (µg/kg diet)...	125	250	125	250	125	250	125	250	
Growth variables									
Feed intake† (kg/d)	2.52	2.44	2.49	2.53	2.48	2.29	2.28	2.26	0.073
Weight gain† (g/d)	713 ^a	720 ^a	797 ^{ab}	770 ^{ab}	819 ^b	718 ^{ab}	763 ^{ab}	702 ^a	22.5
Feed: gain ratio†	3.54 ^b	3.41 ^{ab}	3.13 ^{ab}	3.28 ^{ab}	3.04 ^{ab}	3.22 ^{ab}	3.00 ^a	3.22 ^{ab}	0.114
Final body weight (kg)	105	108	114	111	118	106	112	105	2.8
Serum concentration									
Day 56									
SCN ⁻ † (µmol/l)	<14 ^a	<14 ^a	24 ^a	62 ^b	77 ^{bc}	104 ^{cd}	155 ^e	128 ^{de}	10.4
T ₄ † (nmol/l)	49 ^{ab}	49 ^{ab}	46 ^{ab}	44 ^{ab}	46 ^{ab}	41 ^{ab}	36 ^b	49 ^{ab}	3.8
T ₃ †† (nmol/l)	1.6 ^{ab}	1.3 ^a	1.4 ^a	1.6 ^{ab}	1.4 ^a	1.6 ^{ab}	2.0 ^b	1.6 ^a	0.13
End of experiment									
SCN ⁻ † (µmol/l)	<14 ^a	<14 ^a	<14 ^a	76 ^b	83 ^b	78 ^b	138 ^c	135 ^c	15.2
T ₄ †† (nmol/l)	56 ^{bc}	55 ^{bc}	55 ^{bc}	48 ^{abc}	50 ^{abc}	41 ^{ab}	38 ^a	57 ^c	3.8
T ₃ (nmol/l)	1.9	1.7	1.6	1.6	1.6	1.5	1.6	1.8	0.13
Thyroid variables									
Weight									
Total†† (g)	9.7 ^{ab}	6.3 ^a	8.8 ^{ab}	10.5 ^{ab}	9.8 ^{ab}	19.2 ^c	15.7 ^{bc}	15.5 ^{abc}	1.77
Relative†† (mg/kg body wt)	93 ^{ab}	58 ^a	77 ^a	95 ^{ab}	83 ^{ab}	164 ^b	139 ^b	147 ^b	18.3
Iodine content††§ (µg/g)	636 ^d	1110 ^c	456 ^{cd}	270 ^{ab}	526 ^{cd}	194 ^a	170 ^a	270 ^{ab}	54.7
Total†† (mg)	6.2 ^{abc}	7.0 ^{bc}	4.0 ^{ab}	3.0 ^{ab}	5.2 ^{ab}	3.8 ^{ab}	2.8 ^a	4.3 ^{ab}	0.85

SCN⁻, thiocyanate ions; T₄, thyroxine; T₃, triiodothyronine.
^{a,b,c,d,e} Mean values within a row not sharing a common superscript letter were significantly different, *P* < 0.05 (Newman-Keuls test).
 * For details of diets and procedures, see Table 2 and pp. 948-952.
 † Significant effect of glucosinolates by two-way ANOVA, *P* < 0.05.
 ‡ Significant effect of iodine by two-way ANOVA, *P* < 0.05.
 § Significant glucosinolate × iodine interaction by two-way ANOVA, *P* < 0.05.

Table 5. *Expt 2. Responses of pigs (initial body weight 22.5 kg) to graded levels of rapeseed press cake in the diet over 111 d*
(Mean values for five pigs per group, with their pooled standard error)

	0		50		100		150		SEM	
	125	250	125	250	125	250	125	250	125	250
Rapeseed press cake, untreated (g/kg diet)...	0	0	50	50	100	100	150	150		
Glucosinolates content (nmol/kg diet)...	<0.1	<0.1	0.8	0.8	1.6	1.6	2.5	2.5		
Iodine dosage ($\mu\text{g}/\text{kg}$ diet)...	125	250	125	250	125	250	125	250	125	250
Growth variables										
Feed intake (kg/d)	2.30	2.52	2.45	2.37	2.46	2.42	2.36	2.35	2.36	2.35
Weight gain (g/d)	744	771	820	761	754	761	741	712	741	712
Feed: gain ratio	3.08	3.26	3.00	3.12	3.25	3.17	3.20	3.30	3.20	3.30
Final body weight (kg)	105	108	114	107	106	107	105	102	105	102
Serum concentration										
Day 56										
SCN ⁻ † ($\mu\text{mol}/\text{l}$)	19 ^a	20 ^a	84 ^b	82 ^b	153 ^c	145 ^c	153 ^c	148 ^c	153 ^c	148 ^c
T ₄ †† (nmol/l)	56 ^{cd}	62 ^d	53 ^{bcd}	58 ^d	47 ^{abc}	56 ^{cd}	38 ^a	43 ^{ab}	38 ^a	43 ^{ab}
T ₃ † (nmol/l)	1.7 ^{ab}	1.8 ^b	1.4 ^{ab}	1.5 ^{ab}	1.6 ^{ab}	1.4 ^{ab}	1.5 ^{ab}	1.3 ^a	1.5 ^{ab}	1.3 ^a
End of experiment										
SCN ⁻ †† ($\mu\text{mol}/\text{l}$)	23 ^a	27 ^{ab}	61 ^{cde}	47 ^{bc}	67 ^c	50 ^{cd}	65 ^{de}	47 ^{bc}	65 ^{de}	47 ^{bc}
T ₄ † (nmol/l)	51 ^b	51 ^b	56 ^b	54 ^b	44 ^{ab}	55 ^b	36 ^a	46 ^{ab}	36 ^a	46 ^{ab}
T ₃ (nmol/l)	1.4	1.3	1.4	1.4	1.4	1.5	1.3	1.2	1.3	1.2
Thyroid variables										
Weight										
Total†† (g)	7.1 ^a	7.7 ^a	12.2 ^{ab}	11.7 ^{ab}	15.4 ^{bc}	12.0 ^{ab}	19.3 ^c	21.3 ^c	19.3 ^c	21.3 ^c
Relative†† (mg/kg body wt)	69 ^a	71 ^a	107 ^{ab}	109 ^{ab}	145 ^{bc}	112 ^{ab}	186 ^{cd}	207 ^d	186 ^{cd}	207 ^d
Iodine content††§ (μg/g)	551 ^d	1130 ^a	306 ^{ab}	481 ^{cd}	224 ^{ab}	363 ^{bc}	170 ^a	256 ^{ab}	170 ^a	256 ^{ab}
Total†† (mg)	4.0 ^a	8.7 ^b	3.9 ^a	5.5 ^a	3.4 ^a	4.4 ^a	3.4 ^a	5.6 ^a	3.4 ^a	5.6 ^a

SCN⁻, thiocyanate ions; T₄, thyroxine; T₃, triiodothyronine.

^{a,b,c,d,e} Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$ (Newman-Keuls test).

* For details of diets and procedures, see Table 3 and pp. 948-952.

† Significant effect of glucosinolates by two-way ANOVA, $P < 0.05$.

‡ Significant effect of iodine by two-way ANOVA, $P < 0.05$.

§ Significant glucosinolate \times iodine interaction by two-way ANOVA, $P < 0.05$.

A dietary level of 100 g rapeseed/kg increased thyroid weight significantly, particularly in animals receiving a low I dosage. In the group receiving 150 g rapeseed/kg diet, the thyroid weight was increased irrespective of I dosage or treatment. The I concentration in thyroid tissue varied between groups from $<200 \mu\text{g/g}$ to $>1000 \mu\text{g/g}$. Rapeseed or glucosinolates enlarged the organ, resulting in a dilution effect, leading to a lower I concentration in the thyroid.

In total thyroid the deposition of I was significantly decreased by glucosinolates indicating a higher fall of I concentration than degree of thyroid enlargement. The 250 $\mu\text{g I/kg}$ diet dosage led to significantly higher deposition of I in the thyroid than the dosage of 125 $\mu\text{g I/kg}$ diet.

Rapeseed press cake (Expt 2)

Neither the amount of press cake in feed, nor the I dosage had a significant effect on feed intake, daily live-weight gain or feed efficiency (Table 5).

As in Expt 1, dietary glucosinolates led to increased serum SCN^- concentrations (Table 5). Also in Expt 2 (Table 5) the SCN^- response did not markedly depend on the quantity of the rapeseed product applied. In blood samples taken at slaughter (day 111) serum SCN^- concentration was hardly increased by rapeseed press cake and no difference was found between groups with different quantities of rapeseed press cake in their diets. Possibly, the longer period of feed withdrawal lowered glucosinolates in the intestines and blood. The significantly higher serum SCN^- concentration of the low I group points to retarded SCN^- elimination, i.e. excretion.

As in Expt 1 (Table 4), in Expt 2 (Table 5) glucosinolates decreased and supplementary I increased serum T_4 concentration.

At day 56, the serum T_3 concentration decreased due to a high proportion of rapeseed press cake in the diet (150 g/kg diet), in contrast to Expt 1 (Table 4), where dietary glucosinolates increased serum T_3 concentration at the first blood sampling. Increasing amounts of rapeseed press cake significantly increased the weight of the thyroid (Table 5). The I-dosage effect of Expt 1 (Table 4) was not confirmed.

I concentration and total thyroidal I deposition responded significantly to I administration and rapeseed press cake (Table 5). There was no difference in total thyroidal I content between the different rapeseed press cake groups.

Moist heat treatment of rapeseed (Expt 3) and rapeseed press cake (Expt 4)

In Expt 3 ANOVA showed that the effect of treatment, i.e. glucosinolate loss, on feed intake and weight gain was significant (Table 6). Weight gain diminution was significant only in the group receiving untreated rapeseed and 250 μg supplementary I/kg. However, this significant result with the higher I dosage seems to be an artefact. In Expt 4 no effect of diets on feed intake, growth intensity and feed:gain ratio was detected.

Treatment of rapeseed and rapeseed press cake hardly affected the serum SCN^- concentration at all. There was a significantly lower SCN^- concentration due to lower glucosinolate intake only at day 56 in Expt 3; in Expt 4 there was a tendency towards this ($P=0.07$) at slaughter. At this blood sampling the I dosage influenced the serum SCN^- status significantly. An increased serum SCN^- concentration in pigs receiving the lower I dosage was also found in Expt 2 (Table 5).

The higher I dosage significantly increased the serum T_4 concentration at each time of blood sampling (Table 6). The response of T_4 concentration to dietary glucosinolate level

Table 6. Responses of pigs to diets containing 150 g/kg rapeseed (Expt 3) or rapeseed press cake (Expt 4) either untreated or moist-heat treated, and containing different levels of iodine*
(Mean values for four pigs per group (Expt 3) or five pigs per group (Expt 4))

	Rapeseed				Rapeseed press cake				
	Untreated 2.7		Treated 0.2		Untreated 2.5		Treated <0.1		
	125	250	125	250	125	250	125	250	
Glucosinolates (mmol/kg diet)...									SEM
Iodine dosage (µg/kg diet)...									
Growth variables									
Feed intake (kg/d)	2.28†	2.26†	2.38†	2.42†	2.36	2.35	2.27	2.45	0.084
Weight gain (g/d)	764 ^{bt}	702 ^{at}	778 ^{bt}	787 ^{bt}	741	712	731	803	26.8
Feed: gain ratio	3.00	3.22	3.05	3.08	3.20	3.30	3.13	3.06	0.94
Final body weight (kg)	112 ^{bt}	105 ^{at}	113 ^{at}	115 ^{bt}	105	102	104	112	3.2
Serum concentration									
Day 56									
SCN ⁻ (µmol/l)	155 ^{bt}	128 ^{abt}	106 ^{abt}	94 ^{at}	153	148	116	142	16.8
T ₄ (nmol/l)	36 ^{at}	49 ^{bt}	36 ^{at}	51 ^{bt}	38 ^{at}	43 ^{at}	44 ^{at}	54 ^{bt}	3.0
T ₃ (nmol/l)	2.0	1.6	1.6	1.7	1.5	1.3	1.6	1.4	0.10
End of experiment									
SCN ⁻ (µmol/l)	138	135	112	107	65 ^{bt}	47 ^{at}	50 ^{at}	44 ^{at}	4.6
T ₄ (nmol/l)	38 ^{at}	57 ^{bt}	39 ^{at}	56 ^{bt}	36 ^{at}	46 ^{abt}	45 ^{abt}	52 ^{bt}	3.4
T ₃ (nmol/l)	1.6	1.8	1.8	1.8	1.3	1.2	1.4	1.3	0.10
Thyroid variables									
Weight									
Total (g)	15.7	15.5	15.9	13.7	19.3 ^{BC†}	21.3 ^{C†}	12.4 ^{AB†}	10.6 ^{A†}	2.76
Relative (mg/kg body weight)	139	147	141	119	186 ^{B†}	207 ^{B†}	119 ^{A†}	94 ^{A†}	25.0
Iodine content (µg/g)	170 ^{at†}	270 ^{abt†}	239 ^{at†}	448 ^{bt†}	170 ^{at†}	256 ^{at†}	275 ^{at†}	503 ^{bt††}	37.6
Total (mg)	2.8	4.3	3.8	6.4	3.4†	5.6†	3.5†	5.5†	1.0

SCN⁻, thiocyanate ions; T₄, thyroxine; T₃, triiodothyronine.
^{a,b} Expt 3: mean values within a row not sharing a common superscript letter were significantly different, *P* < 0.05.
^{A,B,C} Expt 4: mean values within a row not sharing a common superscript letter were significantly different, *P* < 0.05.
 * For details of diets and procedures, see Tables 2 and 3, and pp. 948-952.
 † Significant effect of glucosinolates by two-way ANOVA, *P* < 0.05.
 ‡ Significant effect of iodine by two-way ANOVA, *P* < 0.05.

was significant only in Expt 4 at both blood sampling times. Glucosinolates and I did not affect T₃ status. There was a tendency towards higher serum T₃ concentrations in the low I dosage groups in Expt 4 (day 56, $P=0.06$).

In Expt 4 thyroid weight corresponded to dietary glucosinolate content. No effect of glucosinolates on the weight of the thyroid could be detected in Expt 3. In neither of the two experiments did the I dosage influence thyroid weight.

Both dietary glucosinolate content and I dosage had a significant effect on thyroidal I concentration. The total thyroidal I content was only affected by the I dosage (Expt 3 tendency $P=0.10$, Expt 4 $P=0.04$). The thyroidal I store did not reflect different glucosinolate intakes.

DISCUSSION

The glucosinolate content of rapeseed used (Table 1, Expt 1) was somewhat above 30 mmol/kg defatted air-dried matter (basis 910 g/kg DM) which is the borderline of 00 rapeseed quality proposed already some time ago (Fenwick, 1984). However, among the winter rapeseed types now grown in East Germany, it belongs to the top 30% in terms of glucosinolate content.

The rapeseed press cake used in Expt 2 (Table 1) contained less glucosinolates/kg defatted DM than rapeseed used in Expt 1.

The rapeseed from which the rapeseed press cake was made contained 14.4 mmol glucosinolates/kg DM. Pressing one quarter of the seed as fat (ether extract of DM: rapeseed 437 g/kg → rapeseed press cake 180 g/kg) resulted in a 28% increase in the glucosinolate content of the residue. The different fat contents of rapeseed and rapeseed press cake caused the differences in glucosinolate content. On a fat-free DM basis the glucosinolate contents of seed and cake were almost the same (23.9 and 22.6 mmol/kg respectively).

Moreover, in further investigations, pressing (Keith & Bell, 1991) or grinding (Schöne *et al.* 1994b) of rapeseed did not decisively change the glucosinolate content on a fat-free basis. Addition of water causes degradation of glucosinolates, most probably by activation of endogenous myrosinase. After heat drying, isothiocyanates become volatile. These and further glucosinolate degradation products (nitriles, oxazolidinethiones) were not detectable in moist-heat-treated ground rapeseed and rapeseed press cake (Table 1).

Dose responses

Feed intake and growth. Looking at the experiments with graded rapeseed or rapeseed cake content, only in Expt 1 did the group receiving the highest level of rapeseed (150 g/kg diet) show lower feed intake and growth intensity compared with the group receiving 50 g/kg diet. There was a similar difference between these two rapeseed cake levels (Expt 2) with regard to weight gain. However, this was not significant because of the higher variance of weight gain in this experiment (Expt 2) than in Expt 1, presumably due to the more difficult adaptation of initially lighter animals to the grower diet in Expt 2.

Undoubtedly glucosinolates affect feed intake and growth; as a rule >10 mmol/kg diet resulted in significant impairments in pig experiments (Lüdke & Schöne, 1988). At such dietary levels, glucosinolates definitely act as toxicants. Additional I or thyroid hormone administration is without any effect (Schöne *et al.* 1990; Spiegel *et al.* 1993). Feed intake and growth are restored only by decreasing the glucosinolate load. The response of animals to diets with a lower glucosinolate content is uncertain. Contrary to the results obtained in

Expt 1, in an earlier pig experiment rapeseed meal diet containing 6 mmol glucosinolates/kg had no effect on feed intake and growth (Schöne *et al.* 1991b). Production of rapeseed meal includes a desolventizing-toasting step. This steam heat treatment at least partially destroys sinapine, tannins and possibly further unfavourable substances in rapeseed meal. In rapeseed or rapeseed press cake these substances are present, and together with minor quantities of glucosinolates they could reduce feed intake.

There are some efforts to attribute the lower feed intake to bitter taste and pungent odour of glucosinolate cleavage products. Indeed, human subjects detect bitterness in 'traces' of glucosinolates (Fenwick *et al.* 1983). In preference tests pigs offered rapeseed meal diets whose glucosinolate content differed only by 1 mmol/kg, selected the one with the lower dietary glucosinolate level (Schöne *et al.* 1991a). According to these findings, animals perceive very small quantities of glucosinolates. But this ability has no consequence under normal circumstances and the present experimental conditions, in which animals received one type of feed only and maintained a level of feed intake and growth despite the higher dietary glucosinolate concentration (Bille *et al.* 1983; Rundgren, 1983; Schöne *et al.* 1991b).

Serum thiocyanate concentration. In accordance with the findings of earlier experiments with rapeseed meal on different animal species (Paik *et al.* 1980; Schöne *et al.* 1990), rapeseed and rapeseed press cake drastically increased the serum SCN^- concentration. There are two sources of SCN^- . First, SCN^- is a minor product of glucosinolate degradation by microbial thioglucosidases in the intestine (Oginsky *et al.* 1965). Second, nitriles from glucosinolate degradation release CN^- , which is detoxified by thiosulfate S transferases (rhodanese, EC 2.8.1.1) forming SCN^- .

In the present and in earlier experiments (Tables 4, 5 and 6; Schöne *et al.* 1990) no reproducible dose-response effect of dietary glucosinolate level on serum SCN^- concentration was detected. A better dose relationship was found between glucosinolate intake and urinary SCN^- excretion (Schöne & Paetzelt, 1987). In these balance experiments with pigs, up to 9% of molar glucosinolate intake was found as SCN^- in urine. Further metabolites of glucosinolate degradation (i.e. detoxication), hippuric acid and phenyl acetic acid, were detected in the urine of rats (Lange *et al.* 1992).

Serum thyroid hormone concentration, weight and iodine content of thyroid. In the present experiment and in earlier experiments with pigs (Schöne *et al.* 1990, 1991a,b) glucosinolates decreased serum T_4 concentration, whereas I increased it. Even at the highest dietary glucosinolate levels tested (Expt 1: 2.7 mmol/kg diet, Expt 2: 2.5 mmol/kg diet) the decrease of serum T_4 concentration could be prevented (Table 4) or retarded (Table 5) by more I. In earlier pig experiments (Schöne *et al.* 1990) with rapeseed meal diets containing 10 mmol glucosinolates/kg, at least 500 μg supplementary I/kg diet was required to bring the serum T_4 concentration to the level found in glucosinolate-free controls. However, exceedingly high dietary glucosinolate levels prevent sustained thyroidal hormone production, thereby lowering serum T_4 concentration despite administration of high I doses. Thus 1000 μg supplementary I/kg diet could not compensate for the fall in serum T_4 concentration caused by 19 mmol glucosinolates/kg diet (Schöne *et al.* 1991a).

Serum T_3 concentration was less affected or unaffected by dietary glucosinolates and I dosage in the present and in earlier experiments (Schöne *et al.* 1990, 1991a). The source of T_3 is T_4 which is deiodized mainly in the liver and peripheral tissue. There are findings of an elevated serum T_3 concentration due to I deficiency or antithyroid agents. As described for the rat (Rognoni *et al.* 1982) or chicken (Schöne *et al.* 1991a) the thyroid-stressed pig

shifts the production of T_4 to that of T_3 . T_3 is more potent than T_4 and T_3 has one I atom less than T_4 so that preference for T_3 spares I.

The weight of the thyroid was shown to be a sensitive indicator of dietary glucosinolate levels (Tables 4 and 5). The lower I dosage (125 $\mu\text{g}/\text{kg}$ diet) seemed to be almost sufficient. In earlier pig and chicken experiments (Schöne *et al.* 1991a) with higher dietary glucosinolate levels (0–19 mmol/kg) or I doses (0–1000 $\mu\text{g}/\text{kg}$ diet), the effect of I in impeding an increase in thyroid weight or the development of goitre was significant.

Generally, grain diets without the addition of I or without inclusion of alternative I sources such as fishmeal, deplete the I store of the thyroid and produce goitre. Diets containing rapeseed and glucosinolates accelerate this process. More I counteracts these antithyroid effects, however, only up to a certain dietary glucosinolate level. In the present experiments 250 μg supplementary I/kg diet prevented thyroid enlargement of animals receiving up to about 1.5 mmol glucosinolates/kg diet (Tables 4 and 5). In pig experiments with rapeseed meal (Schöne *et al.* 1991b), the same I dosage counteracted the thyroid weight increase induced by an even higher dietary glucosinolate level (2.4 mmol/kg diet). This stronger antidotal effect of I in rapeseed meal may be due to the fact that further goitrogenic agents are removed by toasting.

The effect of glucosinolates on thyroid weight may be explained by the impairment of iodide uptake, its oxidation to I, the I binding to thyroglobulin and synthesis as well as release of hormones, mainly T_4 . Impaired hormone production causes the pituitary to release thyroid-stimulating hormone (TSH) which increases the height of epithelial cells and the weight of the thyroid. It should be noted that in the present experiments glucosinolate-mediated thyroid enlargement might occur without a decrease of serum T_4 concentrations. The TSH release and the effect on thyroid weight depend on the production of hormone. In tracer experiments in pigs, Ingram & Evans (1980) affected the thyroid by altering the supply of dietary energy. Despite the production of different quantities of hormone, no effect on serum T_3 or T_4 concentration was detected.

Moist heat treatment of rapeseed and rapeseed press cake drastically diminished the content of glucosinolates and glucosinolate degradation products. However, the antithyroid action persisted, particularly in the case of rapeseed (Table 6). Heating of ground soaked rapeseed not only causes volatilization of isothiocyanates or nitriles, but also reacts with rapeseed amino acids to form thiourea and, possibly, other strong goitrogens (Langer & Štolc, 1965). Diedrich & Kujawa (1987) suggested some possible reactions of rapeseed matrix compounds with glucosinolate degradation products and reiterated the old demand to determine these antithyroid compounds in rapeseed products.

The highest thyroidal I deposit ($\geq 1000 \mu\text{g}$ I/g fresh weight) was found in pigs receiving diets without glucosinolates (controls) with 250 μg supplemental I/kg diet, which confirms earlier findings (Schöne *et al.* 1990, 1991a). Glucosinolates reduced the thyroidal I deposition, whereas I increased it. The difference between tested dietary levels of glucosinolates was significant (Tables 4, 5, and 6).

In earlier experiments (Schöne *et al.* 1990, 1991a,b) very high glucosinolate levels (10–19 mmol/kg diet) lowered I deposition in the thyroid. Clearly, this statement applies to moderate or high I administration (250 μg –1000 μg supplementary I/kg diet). A lower I dosage causes a low thyroidal I deposition even in diets without antithyroid compounds. The organism needs virtually all the I for hormone production, and storage is quite impossible.

The thyroid is the target of I but also of glucosinolate degradation products. The organ picks up I and oxidizes it via thyroid peroxidase to I, binding to tyrosyl residues. This thyroglobulin-bound I or the I of hormone or hormone precursor represents almost all I in

the thyroid (Taurog, 1985). In the presence of glucosinolates, the action of thyroid peroxidase seems to be modified. According to an *in vitro* experiment by Kohler *et al.* (1988), the enzyme oxidizes oxazolidinethiones, representing important glucosinolate degradation products. This detoxication by thyroid peroxidase consumes elemental I; more exactly, iodine will be reduced to iodide which cannot be taken in by thyroglobulin. Glucosinolates, oxazolidinethiones and further glucosinolate degradation products limit the ability of peroxidase to oxidize iodide to I, resulting in lowering iodization of thyroglobulin and thyroid I deposition, respectively.

In conclusion, pig diets containing ground rapeseed or rapeseed press cake may contain up to 2 mmol glucosinolates/kg. At least 250 µg supplementary I/kg diet is required to prevent a decrease of serum T₄ concentration and thyroid enlargement. Moist heat treatment degrades glucosinolates in ground rapeseed or rapeseed press cake making it possible to incorporate as much as 150 g/kg diet of such treated rapeseed feeds.

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