Dietary low-glucosinolate rapeseed meal affects thyroid status and nutrient utilization in rainbow trout (*Oncorhynchus mykiss*)

Christine Burel¹, Thierry Boujard¹*, Anne-Marie Escaffre¹, Sadasivam J. Kaushik¹, Gilles Boeuf², Koen A. Mol³, Serge Van der Geyten³ and Eduard R. Kühn³

¹Laboratoire de Nutrition des Poissons, Unité mixte INRA-IFREMER, Station d’Hydrobiologie, BP 3, 64310 Saint Pée-sur-Nivelle, France
²Laboratoire de Physiologie des Poissons, IFREMER, Station de Brest, BP 70, 29280, Plouzané, France
³Laboratory of Comparative Endocrinology, Catholic University of Leuven, Naamsestraat 59, 3000 Leuven, Belgium

(Received 29 July 1999 – Revised 29 October 1999 – Accepted 12 November 1999)

Two rapeseed (*Brassica napus*) meals, RM1 and RM2, with two levels of glucosinolates (GLS; 5 and 41 μmol/g DM respectively) were incorporated at the levels of 300 and 500 g/kg of the diets of juvenile rainbow trout (*Oncorhynchus mykiss*) in replacement of fish meal, and compared with a fish-meal-based diet. A decrease in the digestibility of the DM, protein, gross energy and P was observed with high-rapeseed meal (RM) incorporation. In trout fed on RM-based diets, growth performance was reduced even after only 3 weeks of feeding. Feed efficiency was adversely affected by RM and GLS intake. Protein and energy retention coefficients were significantly lower in fish fed on the diet containing the higher level of GLS. P retention was significantly lower with all the RM-based diets than with the fish-meal diet. Irrespective of the degree of growth inhibition, fish fed on RM-based diets exhibited similar typical features of hypothyroid condition due to GLS intake, expressed by lower plasma levels of triiodothyronine and especially thyroxine and a hyperactivity of the thyroid follicles. This hypothyroidal condition led to a strong adjustment of the deiodinase activities in the liver, the kidney and the brain. A significant increase of the outer ring deiodinase activities (deiodinases type I and II respectively) and a decrease of the inner ring deiodinase activity (deiodinase type III) were observed. It is concluded that the observed growth depression could be attributed to the concomitant presence of GLS, depressing the thyroid function, and of other antinutritional factors affecting digestibility and the metabolic utilization of dietary nutrients and energy.

Rainbow trout: Rapeseed: Growth: Glucosinolates: Thyroid function

Rapeseed is primarily grown for its high oil content (400–450 g/kg seed) but the defatted meal is also used as a source of protein for mammals and poultry. Rapeseeds are nevertheless known to contain antinutritional factors, such as a high fibre content, tannins, phytic acid and glucosinolates (anionic β-thio-D-glucopyrranosides encountered in the leaves, seeds, flowers and roots of a large variety of plants, such as rapes). The incorporation of rapeseed meals (RM) containing high levels of glucosinolates (GLS) to animal feed leads to reduced feed intake, enlarged thyroid, reduced plasma thyroid hormone levels and, occasionally, organ abnormalities (liver and kidney) and even mortality (Bunting, 1981; VanEtten & Tookey, 1983).

On account of its good amino acid profile, many studies since the seventies have focused on the incorporation of RM into fish feed (Yurkowski et al. 1978; Teskeredzic et al. 1995; Webster et al. 1997). A comprehensive review has also been made by Higgs et al. (1996). In brief, it has been shown that the deleterious effects of the high levels of fibre and of the GLS restrict the level of incorporation of RM into fish feeds to approximately 200 g/kg (Yurkowski et al. 1978; Hardy & Sullivan, 1983; Hilton & Slinger, 1986; Leatherland et al. 1987; Gomes & Kaushik, 1989; Gomes et al. 1993; Higgs et al. 1996). However, significant improvements have been made to reduce the levels of GLS below 20 μmol/g and to improve the nutritional value of RM by genetic selection of cultivars (Vermorel et al. 1986) and by the use of new processing techniques (Bell, 1993).

Studies carried out by Vermorel & Baudet (1987) in terrestrial animals and by Hardy & Sullivan (1983) in trout have shown that thyroid disturbances (decreasing plasma triiodothyronine (T₃) and thyroxine (T₄) levels) caused by

Abbreviations: ADC, apparent digestibility coefficient; D, deiodinase; IRD, inner ring deiodination; GLS, glucosinolates; ORD, outer ring deiodination; RM, rapeseed meal; rT₃, 3,3',5'-triiodothyronine; T₃, triiodothyronine; T₄, thyroxine.

*Corresponding author: Dr Thierry Boujard, fax +33 (0) 5 59 54 51 52, email boujard@st-pee.inra.fr
RM ingestion occurred at a level of incorporation lower than the level which led to growth depression. Higgs et al. (1982) and Hardy & Sullivan (1983) suspected the existence of a compensatory effect, likely to happen through an adjustment of the deiodinase activities. In all vertebrates, T4 is the main secretory product of the thyroid follicles even though T3 is the biologically active hormone. Outer ring deiodination (ORD) converts T4 into T3 and reverse T3 (3,3',5'-triiodothyronine (rT3)) into 3,5-diiodothyronine (T2). Inner ring deiodination (IRD) converts T4 into rT3 and degrades active T3 to T2. The ORD reaction is the activating pathway of T4 metabolism as it produces T3, while the IRD reaction is considered to be the inactivating pathway as it degrades T4 and inactivates T3 (Kühn et al. 1993). Three types of deiodinases have been characterized in mammals (Leonard & Visser, 1986). Type I deiodinase (D1) can catalyse both ORD and IRD and has a substrate preference for rT3, but can also convert T4 into T3. Type II deiodinase (D2) performs only ORD, and prefers T4 as the substrate. Type III deiodinase (D3) has exclusive IRD activity and deiodinates T3 preferentially. Recent studies have demonstrated that deiodinating activities similar to the mammalian D1, D2 and D3 are also found in five teleosts, including rainbow trout (Oncorhynchus mykiss) (Mol et al. 1998). Generally, in studies with fish, only the hepatic T4 ORD (D2) reaction in liver has been considered, which may neglect an important part of the peripheral thyroid metabolism.

In this present study, two RM with different levels of GLS (5 to 41 mol/kg DM) were incorporated each at two levels (300 and 500 g/kg) in the diets of rainbow trout. The aim of the study was to determine a possible relationship between thyroidal-axis status (plasma levels of thyroid hormones, deiodinase activities, i.e. D1, D2 and D3 in different tissues and the activity of thyroid follicles) and growth and nutrient and energy utilization in rainbow trout.

**Materials and methods**

Two different rapeseed meals (*Brassica napus*) (provided by the Centre Technique Interprofessionnel des Oléagineux Métropolitains (CETIOM), Paris, France) with different levels of GLS were used. Rapeseeds were first subjected to an intense dehulling treatment in order to reduce their fibre content. Two different oil-extraction processes were then used. The first consisted of a pressure-cooking (97°, 5000 kPa) under wet conditions. Any remaining oil was extracted by six consecutive hexane washings in percolation, followed by removal of solvent by steam injection (105°, 600 kPa). Subsequent grinding produced rapeseed meal 1 (RM1). The method used to obtain rapeseed meal 2 (RM2) consisted of a direct oil extraction. Rapeseeds were subjected to a double pressing, and oil was removed by eight consecutive hexane washings. The removal of solvent was done using steam injection (80°, 300 kPa). The thermal treatment applied to RM1 significantly decreased the total GLS level to 5 mol/kg DM, compared with RM2 which contained 41 mol/kg DM (analyses were carried out by the Laboratoire d’Analyse du Cetiom, Orléans, France). Six different GLS were identified in RM1. Of these, progoitrine (2.2 mol/kg DM) and glucobrassicin (1.2 mol/kg DM) were the most important. Ten different GLS were identified in RM2. Progoitrine (17.2 mol/kg DM), gluconapine (6.9 mol/kg DM), glucobrassicin (2.2 mol/kg DM), sinalbine (4.5 mol/kg DM) and glucobrassicine (7.6 mol/kg DM) were the major components. A conversion factor of 0.432, based on the molar weight of each GLS and their average proportion in RM, can be applied to convert the GLS values expressed as mol/kg to g/kg. The chemical composition of these two RM is shown in Table 1. Their amino acid profiles meet the requirements of rainbow trout (Tables 2 and 3).

**Diets and digestibility measurements**

For the growth study, five experimental diets were formulated (Table 3), containing respectively no rapeseed (control diet), 300 and 500 g RM1/kg (R1-300 and R1-500 diets), 300 and 500 g RM2/kg (R2-300 and R2-500 diets).

The apparent digestibility coefficients (ADC) of the diets were measured using the indirect method developed by Choubert (1999) with diets containing Cr2O3 (10 g/kg) as an inert tracer. Ten tanks (60 litres capacity with a flow rate of 5 litres/min, 16–5°) with twenty fish in each (body weight about 100 g each) were adapted to the experimental conditions (12 h light–12 h dark) and fed on one of the experimental diets for 7 d before the start of faecal collection. Faecal samples were collected continuously during 7 d from each tank using the apparatus developed by Choubert et al. (1982). After freeze-drying, the faeces (two samples per

<table>
<thead>
<tr>
<th>Table 1. Chemical composition of the fish meal* and rapeseeded meals†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (g/kg DM)</td>
</tr>
<tr>
<td>Ash (g/kg DM)</td>
</tr>
<tr>
<td>Crude protein (g/kg DM)</td>
</tr>
<tr>
<td>Crude fat (g/kg DM)</td>
</tr>
<tr>
<td>Phosphorus (g/kg DM)</td>
</tr>
<tr>
<td>Starch (g/kg DM)</td>
</tr>
<tr>
<td>N-free extracts (g/kg DM)</td>
</tr>
<tr>
<td>Energy (kJ/kg DM)</td>
</tr>
<tr>
<td>Phylic acid (g/kg DM)</td>
</tr>
<tr>
<td>Glucosinolates (mol/kg DM)</td>
</tr>
</tbody>
</table>

DM, dry matter.
* Norwegian herring meal.
† For details of the oil-extraction processes used to prepare rapeseeded meals 1 and 2, see above.
‡ The amount of N-free extracts (lignin, non-starch polysaccharides, oligosaccharides) was estimated as follows: N-free extracts (g/kg dry matter) = 100 – (ash + crude protein + crude fat + starch).
Table 2. Essential amino acid composition (g/16 g nitrogen) of the fish meal* and rapeseed meal† as compared with the requirements of trout

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Trout requirements‡</th>
<th>Fish meal</th>
<th>Rapeseed meal 1</th>
<th>Rapeseed meal 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg</td>
<td>3.6</td>
<td>5.0</td>
<td>6.8</td>
<td>7.9</td>
</tr>
<tr>
<td>Lys</td>
<td>5.3</td>
<td>5.8</td>
<td>6.6</td>
<td>6.6</td>
</tr>
<tr>
<td>His</td>
<td>1.6</td>
<td>2.5</td>
<td>2.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Ile</td>
<td>2.4</td>
<td>4.3</td>
<td>3.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Leu</td>
<td>4.4</td>
<td>7.3</td>
<td>4.5</td>
<td>6.1</td>
</tr>
<tr>
<td>Val</td>
<td>3.2</td>
<td>5.4</td>
<td>3.7</td>
<td>4.3</td>
</tr>
<tr>
<td>Met</td>
<td>1.8</td>
<td>2.4</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Cys</td>
<td>0.9</td>
<td>0.9</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Phe</td>
<td>3.2</td>
<td>4.0</td>
<td>4.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Tyr</td>
<td>2.0</td>
<td>3.2</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Thr</td>
<td>3.3</td>
<td>3.6</td>
<td>4.9</td>
<td>5.6</td>
</tr>
<tr>
<td>Trp</td>
<td>0.6</td>
<td>1.1</td>
<td>1.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* Norwegian herring meal.
† For details of the oil-extraction processes used to prepare rapeseed meals 1 and 2, see p. 654.
‡ Requirements according to the National Research Council (1993).

Table 3. Ingredients and chemical composition of diets used in the growth experiment

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Control</th>
<th>R1-300</th>
<th>R1-500</th>
<th>R2-300</th>
<th>R2-500</th>
<th>Trout requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapeseed meal 1*</td>
<td>30</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapeseed meal 2*</td>
<td>30</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish meal†</td>
<td>53</td>
<td>35</td>
<td>20.5</td>
<td>35</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Soluble fish protein concentrate</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Flaked maize</td>
<td>32</td>
<td>18</td>
<td>10.5</td>
<td>18</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Fish oil</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>11</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Vitamin mixture‡</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mineral mixture§</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Binder (sodium alginate)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Chemical composition

| Dry matter (g/kg) | 940 | 942 | 942 | 933 | 941 |
| Ash (g/kg DM)     | 104 | 92  | 82  | 92  | 83  |
| Phosphorous (g/kg DM) | 15 | 15  | 14  | 15  | 14  |
| Crude protein (g/kg DM) | 400 | 402 | 387 | 402 | 389 |
| Arg                | 19   | 22   | 23   | 23   | 25   | 14   |
| Lys                | 22   | 24   | 24   | 24   | 24   | 21   |
| His                | 10   | 10   | 10   | 11   | 11   | 6    |
| Ile                | 17   | 17   | 15   | 17   | 17   | 10   |
| Leu                | 27   | 27   | 23   | 29   | 26   | 18   |
| Val                | 19   | 19   | 17   | 20   | 19   | 13   |
| Met                | 9    | 9    | 8    | 9    | 9    | 7    |
| Cys                | 4    | 6    | 7    | 6    | 7    | 4    |
| Phe                | 16   | 16   | 15   | 16   | 15   | 13   |
| Tyr                | 12   | 14   | 14   | 14   | 15   | 8    |
| Thr                | 14   | 16   | 17   | 17   | 18   | 13   |
| Trp                | 4    | 5    | 5    | 5    | 5    | 2    |
| Crude fat (g/kg DM) | 161 | 169 | 171 | 159 | 158 |
| Starch (g/kg DM)   | 228  | 123  | 722  | 125  | 562  |
| N-free extracts (g/kg DM)† | 107 | 214 | 288 | 222 | 314 |
| Gross energy (kJ/kg DM)‡ | 2140 | 2190 | 2200 | 2190 | 2230 |
| Digestible protein (g/kg DM)¶ | 356 | 363 | 349 | 362 | 336 |
| Digestible energy (kJ/kg DM)¶ | 180 | 182 | 179 | 178 | 174 |
| Digestible protein: digestible energy value (mg/kJ) | 19-7 | 19-9 | 17-9 | 17-8 | 17-4 |
| Glucosinolates (mol/kg DM)** | – | 1.4 | 2.3 | 11.6 | 19.3 |

DM, dry matter.
* For details of the oil-extraction processes used to prepare rapeseed meals 1 and 2, see p. 654.
† Norwegian herring meal, 700 g crude protein/kg.
‡ National Research Council (1993).
§ Mineral mixture contained (kg diet): calcium carbonate 1.12 g, magnesium oxide 0.62 g, ferric citrate 0.1 g, potassium iodide 0.2 mg, zinc sulfate 0.2 g, copper sulfate 0.15 g, manganese sulfate 0.15 g, dibasic calcium phosphate 2.5 g, cobalt sulfate 1 mg, sodium selenite 1.5 mg, KG 0.45 g, NaCl 0.2 g.
¶ Includes lignin, non-starch polysaccharides and oligosaccharides and were estimated as follows: N-free extracts (g/kg) = 100 – (ash + crude protein + crude fat + starch).
†† Calculated from apparent digestibility coefficient data of the digestibility experiment (Table 4).
** Calculated from the glucosinolate content of the rapeseed meals (see Table 1).
diet, each sample corresponding to a pool of 7 d of defecation from each tank) were analysed for $\text{Cr}_2\text{O}_3$, protein, fat, energy, ash and P content. The ADC values of DM, nutrients and energy in the different experimental diets were calculated according to Maynard & Loosly (1969):

$$\text{ADC of DM} = 1 - \left( \frac{\text{dietary } \text{Cr}_2\text{O}_3}{\text{faecal } \text{Cr}_2\text{O}_3} \right)$$

$$\text{ADC of nutrient or energy} = 1 - \left( \frac{\text{dietary } \text{Cr}_2\text{O}_3}{\text{faecal } \text{Cr}_2\text{O}_3} \right) \times \left( \frac{\text{faecal nutrient or energy/dietary nutrient or energy}}{1} \right)$$

**Growth study**

Fifteen outdoor tanks (500 litres) were used at the INRA experimental fish farm (Donzacq, Landes, France) during spring 1995. Tanks were supplied with spring water ($17 \pm 1^°\text{C}$, flow rate 10 litres/min). Fifty rainbow trout with an average body weight of 20 g were randomly allocated to each tank. After a 1-week adaptation period, one of the five diets were fed to each group of three tanks of fish for 9 weeks. Food was distributed twice a day by hand, to apparent satiety. Fish were weighed and the voluntary feed intake was recorded every 3 weeks.

At the end of the growth study, a series of tissue and blood samplings were performed on fish fed on the respective diets. In order to minimize the possibility of time and tank effects, samples of fish were taken from the tanks at two different times over a 4-d period (from day 70 until day 73), according to a randomly scheduled sequence. In addition, to keep disturbance to the fish at minimum, the same tanks were never sampled more than once every 24 h and nocturnal samplings were conducted without using any light. In total, this series of sampling resulted in five fish per tank 8 h before the morning meal (at 00.00 hours) and five others 4 h after the morning meal (at 12.00 hours), i.e. thirty fish per dietary treatment.

Blood samples of all sampled fish were obtained from vessels near the caudal peduncle using a heparinized syringe. Blood was immediately centrifuged and plasma was stored at $-20^°\text{C}$. The liver, the brain and the kidney of all sampled fish were dissected following blood sampling, frozen in liquid N$_2$, and stored at $-80^°\text{C}$. Ten additional fish were taken on day 73 at 12.00 hours from both control and the $R_2$ treatment, while $D_2$ activity was measured in the liver ($R_3$ activity was performed after orthophthaldehyde (Sigma P 1378, St Quentin, France) derivatization of amino acids using HPLC (HPLC-Varian Model 5000, Varian, Limerick, Ireland, C$_{18}$ Aminotag column, Varian, Limerick, Ireland) following a modified procedure of Gardner & Miller (1980).

**Thyroid status**

**Assay for thyroid hormones in plasma.** Plasma thyroid hormone ($T_4$ and $T_3$) levels were measured with a radioimmunoassay described by Boeuf & Prunet (1985) and modified by Martinez et al. (1995). The detection limits for the $T_4$ and $T_3$ radioimmunoassays were 1.25 and 0.62 ng/ml respectively. The specific binding obtained was 46 % for $T_4$ and 67 % for $T_3$, and the non-specific binding was 10 % for $T_4$ and 9 % for $T_3$. These percentages were estimated at a hormone level of 80 ng/ml. The intra-assay CV was 10 % for both $T_4$ and $T_3$.

**Deiodinase assay.** A previous study (Mol et al. 1998) allowed us to determine the different types of deiodinases present in rainbow trout ($D_1$, $D_2$ and $D_3$) and the tissues with the highest level of enzymic activity (liver, brain and kidney). Deiodinase activities were measured as described in that paper.

The microosomal fractions were prepared as described previously (Mol et al. 1998). Tissues were homogenized in five volumes of buffer (0.25 M-sucrose, 10 mM-HEPES, pH 7.0, 1 mM-1,4-dithiothreitol) and centrifuged for 20 min (4° at 25000 g). The supernatant fraction was then centrifuged for 60 min (4° at 100 000 g). The resulting pellet and fluffy upper layer were resuspended together in three volumes of buffer (0.1M-phosphate, pH 7.0, 2 mM-EDTA, 1 mM-1,4-dithiothreitol), snap-frozen in portions and stored at $-80^°\text{C}$. All procedures were carried out on ice. Protein concentrations were determined with the BCA protein assay reagent (Bio-Rad, Nazareth, Belgium) using bovine serum albumin as a standard.

$D_1$ activity was measured in the kidney ($n$ 30 per dietary treatment), while $D_2$ activity was measured in the liver ($n$ 30 per dietary treatment) and $D_3$ activity was measured in the brain. In brief, an adequate amount of microsomal protein (1 mg/ml for each assay except in the case of liver $D_2$: 0.25 mg/ml) suspended in sodium phosphate buffer (0.1 M, 2 mM-EDTA, 1 mM-1,4-dithiothreitol, pH 7.1) was incubated with a similar amount of substrate–cofactor solution (kidney $D_1$ and liver $D_2$: 1 h at 30°; brain $D_3$: 1 h at 37°). 3.70 kBq of the preferred radioactive (125I) substrate was added in the substrate–cofactor solution ($D_1$: rT$_3$ (0.1mM); $D_2$: $T_4$ (1 nM); $D_3$: $T_3$ (10 nM)) and 1,4-dithiothreitol as cofactor.
(30 mM for D1; 50 mM for D2; 10 mM for D3 in brain). Each sample was tested in duplicate, together with blanks, containing no protein, to measure non-enzymic degradation of the tracer. For the D1 and D2 enzyme assays, the reaction was stopped on ice by addition of bovine serum albumin (50 g/l) and release of radioiodine by ORD of outer ring-labelling rT3 or T4 was estimated (γ-counter, Hewlett-Packard, Groningen, The Netherlands) after precipitation of protein-bound iodothyronines with TCA (100 g/l). For the D3 assay, reactions were stopped by addition of methanol and iodothyronine products were analysed by HPLC (Eelkman Rooda et al. 1989). The deiodinase activity is expressed as fml substrate converted/mg protein per minute.

Thyroid follicle histology. Lower jaws were kept in Bouin’s fluid for 18 d. Jaws were decalcified in TCA (50 g/l) for 7 d, and after dehydration, the tissues were embedded in paraffin. Serial sections (20 μm) of longitudinally-oriented lower jaws were made. One section out of ten was kept and stained according to Gabe (1968) by alcian blue (pH 2-6), Groat’s haematoxylin and orange G. Histological examinations were made with a semi-automatic image analyser (VIDS-IV, Systèmes Analytiques, Compiègne, France). The volume occupied by the total thyroid follicles was calculated from areas observed on serial sections in seven fish. The quantity of follicles was estimated using a screen (ninety-six equidistant dots; magnification ×60) which was moved over the entire area of each section. The number of dots situated on a follicle was counted and the corresponding volume (mm³) was calculated by:

\[ V = \frac{(N \times S \times E \times C)}{96}, \]

where \( N \) is the total number of the dots counted on the successive sections in each fish, \( S \) is the surface area of the screen (mm²), 96 is number of dots in the screen, \( E \) is the thickness of the sections (mm), \( C \) is the interval between two sections examined (mm).

The mean height (distance between basal and apical face of cell; magnification ×500) of 100 follicle epithelial cells was also measured in ten fish.

Data analysis

The effect of dietary treatments was analysed by one-way ANOVA \((P < 0.05)\), and when appropriate, means were compared by the Tukey’s multiple range test. When only two series of data were compared, the Student’s \( t \) test was performed \((P < 0.05)\). Pearson’s correlation coefficients \((r_s; P < 0.005)\) or regression coefficients \((r^2; P < 0.05)\) were used to assess the relationship between the different variables.

**Results**

Digestibility of components of experimental diets

The ADC of protein and fat were significantly lower in the R2-500 diet than in the other RM-based diets (Table 4). However, it was not different from the control diet. A significant inverse relationship was found between the GLS content of the diet and the ADC of the DM \((r^2 0.97; P < 0.05)\). The ADC of energy was significantly lower in the R2-500 diet compared with all the other diets. The ADC of starch was high (>0.88), and its variation was associated mainly with the dietary starch content \((r^2 0.86; P < 0.05)\). The ADC of P was low in all diets (<0.34) and especially with the R2-500 diet which contained the highest quantity of RM2.

Growth performance, feed intake and feed efficiency

The incorporation of RM in the diet caused a significant decrease in growth performance already after three weeks of feeding (Fig. 1(A)). The voluntary feed intake was adversely affected only over the first 3 weeks of feeding (Fig. 1(B)). After 6 weeks the feed intake of groups fed on the RM-based diet was similar to, or greater than that of fish fed on the control diet. The decrease in daily growth index (Table 5) was accompanied by a decrease in feed efficiency. Taking into account the different levels of dietary incorporation of rapeseed meal, these decreases were stronger with RM2, containing a higher amount of GLS, than with the heat-treated one (RM1). When the daily growth index and the feed efficiency were plotted against GLS intake (see Fig. 2 for feed efficiency), there was a strong decrease even at a low level of GLS intake (30–47 μmol/kg average-fish-body-weight and per d), with a plateau up to an intake level of 242 μmol/kg average-body-weight per d and a further decrease in daily growth index and feed efficiency was observed for higher levels of GLS intake (422 μmol/kg average body weight per d).

**Table 4. Apparent digestibility coefficients of the nutrients and energy in the experimental diets by rainbow trout**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>R1-300</th>
<th>R1-500</th>
<th>R2-300</th>
<th>R2-500</th>
<th>MSE between groups</th>
<th>MSE within groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>0.78</td>
<td>0.76</td>
<td>0.76</td>
<td>0.73</td>
<td>0.69</td>
<td>0.213</td>
<td>0.009</td>
</tr>
<tr>
<td>Protein</td>
<td>0.89</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.86</td>
<td>0.055</td>
<td>0.014</td>
</tr>
<tr>
<td>Fat</td>
<td>0.91</td>
<td>0.94</td>
<td>0.95</td>
<td>0.93</td>
<td>0.86</td>
<td>0.254</td>
<td>0.068</td>
</tr>
<tr>
<td>Gross energy</td>
<td>0.84</td>
<td>0.83</td>
<td>0.81</td>
<td>0.81</td>
<td>0.78</td>
<td>0.109</td>
<td>0.016</td>
</tr>
<tr>
<td>Starch</td>
<td>0.88</td>
<td>0.94</td>
<td>0.99</td>
<td>0.91</td>
<td>0.98</td>
<td>0.418</td>
<td>0.017</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.33</td>
<td>0.33</td>
<td>0.34</td>
<td>0.34</td>
<td>0.15</td>
<td>1.414</td>
<td>0.226</td>
</tr>
</tbody>
</table>

MSE, mean squared error.

\(^{a,b,c}\) Mean values within a row with unlike superscript letters were significantly different (one-way ANOVA and Tukey’s multiple range test; \( P < 0.05 \)).

\(*\) For details of composition of diets see Table 3; the glucosinolate content of the diets were (μmol/kg dry matter): R1-300 1.4, R1-500 2.3, R2-300 11.6, R2-500 19.3.
Protein and energy retention were significantly lower in fish fed on the R2-500 diet than in those fed on the other diets (Table 5). P retention was significantly lower in all fish fed on the RM-based diets than in fish fed on the control diet irrespective of the kind of RM and the level of incorporation. The decrease in P retention was correlated with an increase in RM intake (rs = -0.80; P < 0.001).

**Thyroidal status**

No significant effect of sampling time (diurnal and nocturnal samplings) was detected in plasma levels of T3, T4, and deiodinase activities. Ingestion of RM-based diets induced a significant decrease (about 40%) of the plasma T3 level and an even more important decrease (about 80%) of the plasma T4 level, regardless of the RM used and its level of incorporation (Fig. 3).

As shown in Table 6, the T3 : T4 ratio was considerably higher (about 300%) in fish fed on RM-based diets than in fish fed on the control diet. D2 activity in the liver and D1 activity in the kidney were increased by 600% and 50% respectively, while D3 activity was inhibited in the brain (about 60%) in fish fed on RM-based diets.

Changes in thyroid follicle activity, as determined through histological studies, are shown in Figs 4 and 5 and Table 6. The thyroid follicles were mainly located in the lower jaw of trout, scattered alongside the ventral aorta near the first brachial arches (Fig. 4). The volume of total thyroid tissue was significantly higher in fish fed on the R2-500 diet (about 270%) than in fish fed on the control diet (Fig. 5(A)).

**Table 5. Growth, feed intake, nutrient and energy retention of rainbow trout fed on the experimental diets for 64 d†**

(Mean values and mean squared errors for 150 fish per group)

<table>
<thead>
<tr>
<th>Diets*</th>
<th>Control</th>
<th>R1-300</th>
<th>R1-500</th>
<th>R2-300</th>
<th>R2-500</th>
<th>MSE between groups</th>
<th>MSE within groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth performance and feed utilization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>90.6±</td>
<td>82.5±</td>
<td>71.9b</td>
<td>71.8ab</td>
<td>65.2a</td>
<td>302.4±</td>
<td>13.2</td>
</tr>
<tr>
<td>Biomass gain (% IBW)</td>
<td>284±4</td>
<td>252±2</td>
<td>214±4</td>
<td>208±2</td>
<td>167±2</td>
<td>6033±</td>
<td>208</td>
</tr>
<tr>
<td>Daily growth index‡</td>
<td>2.6±c</td>
<td>2.3±c</td>
<td>2.1b</td>
<td>2.0b</td>
<td>1.8a</td>
<td>0.295±</td>
<td>0.004</td>
</tr>
<tr>
<td>Voluntary feed intake (% of body weight/d)§</td>
<td>2.0±c</td>
<td>2.1bc</td>
<td>2.1ab</td>
<td>2.0bc</td>
<td>2.2c</td>
<td>0.01±6</td>
<td>0.002</td>
</tr>
<tr>
<td>Feed efficiency ratio</td>
<td>0.99±d</td>
<td>0.86±d</td>
<td>0.80±b</td>
<td>0.81bc</td>
<td>0.68±b</td>
<td>0.0374±</td>
<td>0.0009</td>
</tr>
<tr>
<td>Retention (% of intake)¶</td>
<td>Protein</td>
<td>36.0±3</td>
<td>31.7ab</td>
<td>32.5b</td>
<td>32.3b</td>
<td>27.1a</td>
<td>30±5</td>
</tr>
<tr>
<td></td>
<td>Energy</td>
<td>38.6±3</td>
<td>36.6±3</td>
<td>34.9±3</td>
<td>35.6b</td>
<td>27.6a</td>
<td>51±8</td>
</tr>
<tr>
<td></td>
<td>Phosphorus</td>
<td>28.8±3</td>
<td>22.0±3</td>
<td>21.2±3</td>
<td>21.8±3</td>
<td>19.1±a</td>
<td>40±2</td>
</tr>
</tbody>
</table>

MSE, mean squared error; IBW, initial body weight.

Mean values within a row with unlike superscript letters were significantly different (one-way ANOVA and Tukey’s multiple range test; *P < 0.05).§ Voluntary feed intake = 100 × (final body weight – initial body weight)/duration.

† For details of composition of diets see Table 3; the glucosinolate content of diets were (μmol/g dry matter): R1-300 14, R1-500 23, R2-300 11.6, R2-500 19.3.

‡ Daily growth index 100 × (final body weight)1.333 – (initial body weight)1.333/duration.

§ Voluntary feed intake = 100 × dry feed intake (g)/(initial body weight + final body weight) × duration.

¶ Retention = feed intake (g) / [initial body weight × final carcass nutrient content – initial body weight × initial carcass nutrient content]/nutrient intake.
and 5(B). Table 6). The height of the epithelial follicle cells was significantly higher (200%) in fish fed on the R2-500 diet, indicating increased follicle activity (Fig. 5(C) and 5(D)).

**Discussion**

Detailed information on the goitrogenic activity of dietary RM in birds and mammals, including human subjects is available (see Mawson *et al.* 1994b). Data from terrestrial animals as well as from fish (Yurkowski *et al.* 1978; Higgs *et al.* 1982; Hardy & Sullivan, 1983; Hilton & Slinger, 1986; Leatherland *et al.* 1987; Hossain & Jauncey, 1988; Teskeredzic *et al.* 1995; Webster *et al.* 1997) have shown the role of GLS in thyroidal disturbances. In fact, the toxicity is not directly caused by the GLS, but by the GLS breakdown products such as isothiocyanates, thiocyanate anions, oxazolidinethiones and nitriles, mainly as a result of the activity of myrosinase, a specific plant hydrolytic enzyme. In intact plant tissues, the enzyme is stored separately from the GLS substrates in specific cells (idioblasts) (Grob & Matile, 1979). Contact between the two is a result of mechanical injury of the plant tissue as it occurs during the processing of RM.

Our data indicate that the content of intact GLS in the diet in itself may not be a good indicator of the potential deleterious effects of RM in fish. To reduce the GLS content and to denature the myrosinase during the processing of meal, it is necessary to apply high temperatures during the oil-extraction process. The difficulty then is to succeed in the degradation of the GLS without the production of toxic by-products. The two RM used in our study have been

![Fig. 2. Relationship between glucosinolate intake and feed efficiency ratio in rainbow trout fed on diets containing 0, 300 or 500 g rapeseed meal/kg over the whole growth period of 64 d. For details of the oil-extraction procedures used to prepare rapeseed meals 1 and 2 (R1 and R2) see p. 654, and for details of the composition of the diets see Tables 1–3. Each symbol represents one replicate. The diets were: (○), control; (●), R1-300; (●), R1-500; (▲), R2-300; (■), R2-500. No statistical analysis of the three straight lines drawn on the figure was made due to the small number of data.](https://www.cambridge.org/core/coreimage)

![Fig. 3. Plasma concentrations of triiodothyronine (□) and thyroxine (■) in rainbow trout fed on diets containing 0, 300 or 500 g rapeseed meal/kg at the end of the experimental feeding period for each diet. For details of the oil-extraction procedures used to prepare rapeseed meals 1 and 2 (R1 and R2) see p. 654, and for details of the composition of the diets see Tables 1–3. Values are means (n 40 per group) with standard deviations represented by vertical bars. Mean values were significantly different from those of the control group: *P < 0.05* (one-way ANOVA and Tukey’s multiple range test).](https://www.cambridge.org/core/coreimage)

### Table 6. Effects of dietary glucosinolate intake on thyroid function in rainbow trout: thyroid follicular activity, triiodothyronine : thyroxine ratio and peripheral deiodinase activity

<table>
<thead>
<tr>
<th>Diets*</th>
<th>Control</th>
<th>R1-300</th>
<th>R1-500</th>
<th>R2-300</th>
<th>R2-500</th>
<th>MSE between groups</th>
<th>MSE within groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid volume (mm³)</td>
<td>2.8⁻</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>10.3⁻</td>
<td>200</td>
<td>46</td>
</tr>
<tr>
<td>Epithelial cell height (μm)</td>
<td>4.3⁻</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>13.0⁻</td>
<td>339.92</td>
<td>0.57</td>
</tr>
<tr>
<td>D₁ : T₄</td>
<td>0.6</td>
<td>2.3</td>
<td>2.6</td>
<td>2.4</td>
<td>2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D₂ in kidney (fmol T₃)</td>
<td>145.4⁻</td>
<td>224.9⁻</td>
<td>262.0⁻</td>
<td>206.2⁻</td>
<td>232.3⁻</td>
<td>45.8</td>
<td>1.4</td>
</tr>
<tr>
<td>D₂ in liver (fmol T₃)</td>
<td>10.1⁻</td>
<td>72.9⁻</td>
<td>73.2⁻</td>
<td>71.4⁻</td>
<td>72.0⁻</td>
<td>37306</td>
<td>82</td>
</tr>
<tr>
<td>D₃ in brain (fmol T₃)</td>
<td>22.2⁻</td>
<td>8.3⁻</td>
<td>9.3⁻</td>
<td>10.7⁻</td>
<td>6.7⁻</td>
<td>216</td>
<td>43</td>
</tr>
</tbody>
</table>

MSE, mean squared error; ND, not determined; T₃, triiodothyronine; T₄, thyroxine; rT₃, reverse triiodothyronine; D, deiodinase.

* Means within a row with unlike superscript letters were significantly different (one-way ANOVA and Tukey’s multiple range test; *P < 0.05*).  
* For details of the composition of diets see Table 3; the glucosinolate content of the diets were (mol/kg dry matter): R1-300 1.4, R1-500 2.3, R2-300 11.6, R2-500 19.3.

* Thyroid volume was measured on seven fish and the epithelial cell height on ten fish.

* 15, except for deiodinase 3 activity in the brain where *n* 3 (pooled).
Fig. 4. Sagittal section of a rainbow trout head after a period of starvation (1 week; hypothyroidal fish). Large follicles are scattered throughout a highly vascularized region of the lower jaw adjacent to the ventral aorta (see in the square). The histological features suggest a very low activity of the follicles: very large follicles with large, homogenous colloids. va, Section of the ventral aorta; gf, section of gill filaments; oe, oesophagus; m, mouth; e, eye. Magnification ×5.

Fig. 5. Thyroid follicles in rainbow trout. (A) Thyroid follicles in a rainbow trout fed on a control diet for 73 d. The thyroidal tissue is not very dense, the follicles (f) are spread into adipose tissue (at) (magnification ×60). (B) Thyroid follicles of a rainbow trout fed on a diet containing 500 g rapeseed meal (R2)/kg diet (R2-500) for 73 d. Thyroidal tissue is very dense (f) and the proximity to the ventral aorta (va) is evident (magnification ×60). (C) Unstimulated thyroid tissue of a rainbow trout fed on a control diet for 73 d. The follicle epithelial cells (ep) are cuboidal in appearance, and the colloid (c) within the lumen is homogenous. The nucleus : cytoplasm ratio of the epithelial cells is high. Vesiculation (v) of the colloid is evident in this follicle (magnification ×500). (D) Stimulated thyroid tissue of a rainbow trout fed on diet R2-500 for 73 d. The follicle epithelial cells (ep) are very large and are columnar. The colloid (c) is partly or wholly depleted. The nucleus : cytoplasm ratio of the epithelial cells is small (magnification ×500). For details of the oil-extraction procedure used to prepare R2 see p. 654, and for details of the composition of the diets see Tables 1–3.
subjected to two different oil-extraction processes including different thermal conditions. In addition to the different quantities and profiles obtained in GLS, different quantities and profiles of active breakdown products could be obtained either during the process or later on if enough myrosinase remained active (Nugon-Baudon & Rabot, 1994).

In this present study, a deleterious effect of dietary RM incorporation on thyroid status and fish growth is shown, even at an incorporation level of 300 g heat treated RM/kg diet with a very low content of GLS (1-4 μmol/g diet). In the previous studies on fish (Yurkowski et al. 1978; Higgs et al. 1982; Hardy & Sullivan, 1983; Hilton & Slinger, 1986; Leatherland et al. 1987; Hessain & Jauncey, 1988; Teskeredzic et al. 1995; Webster et al. 1997), the dietary amount of toxic compounds was expressed either as the amount of intact GLS, as the amount of intact GLS plus GLS breakdown products or as the amount of GLS breakdown products alone. As a result, the lowest levels of toxic compounds inducing thyroidal disturbance or growth depletion varied greatly between studies. Nevertheless, our results are generally in accordance with those obtained by Yurkowski et al. (1978), Hardy & Sullivan (1983) and Leatherland et al. (1987) in rainbow trout, who determined a critical level of toxic compounds in the range of 1–2 mol/kg diet using different types of processed RM. Amongst terrestrial animals, ruminants are considered to be less sensitive than single-stomached animals (for reviews see Mawson et al. 1994a, b): growth, feed intake and feed conversion are reduced by a dietary GLS content of approximately 3–4 mol/kg diet in the rat, 0.6–3 mol/kg in the young pig, 4–10 mol/kg in growing poultry, and 7–15 mol/kg in young calves; thyroid function is affected by dietary levels of 0.5–4 mol/kg in the rat, 2–3 mol/kg in swine, 1–4 mol/kg in growing poultry, and 8 mol/kg in calves.

Irrespective of the type of RM used, we observed the typical features corresponding to a hypothyroid condition in trout. Lower T1 and T4 levels were accompanied by thyroid tissue hyperactivity, i.e. an increase in the volume of the thyroid tissue and epithelial follicle cell height (hyperplasia and hypertrophy). Moreover, these cells became columnar with the colloid partly or completely depleted. Our results also clearly demonstrate an effect of the hypothyroidal condition on the \textit{in vitro} deiodinase activities. \textit{In vitro} D2 activity in the liver and D1 activity in the kidney were elevated, both offering the possibility for an increased T3 production from the available T4 by ORD. In addition, there was a decrease in \textit{in vitro} D3 activity in the brain, reducing the degradation of T3 by IRD. It is not easy to extrapolate to the \textit{in vivo} situation, but these results suggest that deiodinases compensate for the lack of bioactive T3, as suspected earlier by Higgs et al. (1982) and Hardy & Sullivan (1983) in fish and in terrestrial animals (Mawson et al. 1994b). The \textit{in vivo} potential role of each enzyme in the adjustment of the plasma thyroid hormones levels is difficult to establish, because their activity depends on the substrate and cofactor availability, the intracellular pH and the temperature. Nevertheless, the response of the D3 activity in the brain, given the size of this organ, seems to be a localized response and acts probably to protect the brain against T3 levels which are too low.

The relationship between hypothyroidism and poor growth is established in mammals including human subjects (Boyages et al. 1989; Hetzel, 1994). However, the relationship between the thyroid disturbances induced by the metabolites of GLS and growth performance of fish has not been clearly demonstrated. Indirect evidence was given by Leatherland et al. (1987), who showed that a dietary T3 supplement in trout fed on RM-based diets led to an improved growth performance. In the present study, the lower growth rate of fish fed on the RM-based diets compared with that of the control fish was accompanied by low feed utilization. When this effect is related to dietary GLS intake, data show two thresholds of sensitivity. Indeed, ingestion of very low amounts of GLS, corresponding to a dietary content of GLS of 1-4 mol/kg DM, led to a decrease of both growth rate and feed efficiency, but this effect was not further exacerbated with increasing dietary GLS levels up to 11-6 mol/kg. However, a higher dietary content of GLS (19-3 mol/kg DM) led to a stronger decrease of growth and feed efficiency. But the hypothesis of the existence of these thresholds of sensitivity cannot be assured with the present data, because first, the amount of toxic derivatives of GLS were not measured here, and second, the effect of the other antinutritional factors present in the two rapeseed meals tested must be taken into account. The poor growth performance observed could be due to: (1) thyroid disturbances caused by increased levels of GLS. It is known that thyroid hormones play a role in the regulation of fish growth through intermediary metabolism (metabolic utilization of energy, amino acids and possibly carbohydrates; see Leatherland, 1994). Besides, several authors (Harvey et al. 1988; Luo & McKeown, 1991; Furchi-Pisany et al. 1995; Melamed et al. 1995) have shown a thyroidal regulation of growth hormone metabolism, hypothyroid condition possibly leading to a decrease of GH release. As a matter of fact, a deleterious effect of isolated isothiocyanates on the digestive utilization of nutrients, together with thyroid disturbances, has been reported in carp (\textit{Cyprinus carpio}) (Hossain & Jauncey, 1988). In the rat (Bille et al. 1983; Vermorel & Baudet, 1987; Roland et al. 1996), isolated progoitrine, sinigrin and sinalbine impaired also the thyroid metabolism and protein digestibility and retention. A low level of bioactive T3 may interfere with the digestive capacities of trout, given the effect of a treatment with thyroid hormones on the digestive function of rats (Hodin et al. 1992) and of red sea bream (\textit{Chrysophrys major}) (Woo et al. 1991). In our study, the digestibility of the DM, protein, lipid, energy and P was significantly reduced when the diet contained 500 g untreated RM/kg, i.e. with the higher content of GLS. Nutrient and energy retentions were also reduced in fish fed on this diet. In addition, results of the present study show a dissociation between plasma T3 levels and growth. It seems that there was a threshold of thyroid sensitivity to the GLS breakdown products even at an incorporation level of 300 g heat-treated RM/kg. Two major hypotheses can be formulated. First, the compensatory effects of hypertrophy of thyroidal tissue and of the deiodinase activities may result in sufficient circulating levels in T3 (about 4 ng/ml) to support normal growth. In such case, the goitrogenic activity of GLS breakdown products would play a minor or no role at all in the poor growth performance observed. Second, thyroidal disturbance could...
only be partially responsible for the lower growth rate, and thus, the differential response would be due to the direct
effects of other antinutritional factors; (2) a direct effect of
GLS on feed utilization. Indeed, GLS can interfere with
liver function, because they induce alterations of the hepatic
detoxification system as shown in mammals (Rabot et al.
1993; Williamson et al. 1996). An increase of the weight of
liver and kidneys related to the amount of GLS ingested has
been shown in growing pigs (Bourdon & Aumaître, 1990).
However, no work conducted in fish clearly show this effect,
and in the present study, no liver or kidney abnormality was
observed, especially with the higher level of RM, could be
caused by the concomitant effect of the hypothyroid con-
dition, the direct action of GLS and the action of the other
antinutritional factors.

Acknowledgements

This work has been partially supported by the CETIOM
(Centre Technique Interprofessionnel des Oléagineux
Métropolitains, Paris, France), by the UNIP (Union Nationale
Interprofessionnelle des Plantes Riches en Protéines, Paris,
France) and by the KU Leuven Onderzoeksraad (project
OT/94/11). The authors gratefully acknowledge the skilled
technical assistance of D. Blanc, J. Brèque, Y. Hontang, A.
Le Roux, L. Noterdaeme, P. Peyrotte, F. Sandres, A. Sévère,

References

Incorporation du colza 00 sous forme de tourteau ou d’amande
dans les aliments de la truite arc-en-ciel (Oncorhynchus mykiss):
performance zootechnique et digestibilité (Incorporation of
rapeseed 00, in the form of cattle cake, or almond in diets for
rainbow trout (Oncorhynchus mykiss): zootechnical
performance and digestibility). Bulletin Français de la Pêche et de
la Pisciculture 317, 50–57.

Bell JM (1993) Factors affecting the nutritional value of canola
697.

Bille N, Eggum BO, Jacobsen I, Olsen O & Sorensen H (1983)
Antinutritional and toxic effects in rats of individual glucosino-
lates (+/- myrosinases) added to a standard diet. Zeitschrift für
Tierphysiologie, Tierernährung und Futtermittelkunde 49, 195–
210.

activity and plasma thyroid hormones during smoltification in

Bolin DW, King RP & Klosterman EW (1952) A simplified
method for the determination of chromic oxide (Cr₂O₃) when
used as an index substance. Science 116, 634–635.

Bourdon D & Aumaître A (1990) Low-glucosinolate rapeseeds and
rapeseed meals: effect of technological treatments on chemical
composition, digestible energy content and feeding value for
growing pigs. Animal Feed Science and Technology 30, 175–
191.

Boyages SC, Collins JK, Maherly GF, Jupp JJ, Morris J & Eastman
CJ (1989) Iodine deficiency impairs intellectual and neuromotor
development in apparently normal persons: a study of rural
inhabitants of north-central China. Medical Journal of Australia
150, 676–682.

Bunting ES (editor) (1981) Production and Utilization of Protein

Choubert G (1999) La digestibilité des nutriments chez les poissons:
aspects de méthodologie. (Digestibility of nutrients by fish:
methodological considerations). Cybium 23, Suppl.,
113–125.

fish: improved device for the automatic collection of feces.
Aquaculture 29, 185–189.

Davies NT & Reid H (1979) An evaluation of the phytate, zinc,
copper, iron and manganese contents of, and Zn availability
from, soya-based textured-vegetable-protein meat-substitutes or

Eelkman Rooda SI, Otten MH, van Loon MAC, Kaptein E &
Visscher TJ (1989) Metabolism of triiodothyronine in rat hepatocy-
es. Endocrinology 125, 2187–2197.

Farchi-Pisansy O, Hackett PBJ & Moav B (1995) Regulation of fish
growth hormone transcription. Molecular Marine Biology and
Biotechnology 4, 215–223.


Gabe M (1968) Techniques Histologiques (Techniques in Histol-

Gardner WS & Miller WH (1980) Reverse-phase liquid chromato-
ography of amino acids after reaction with o-phenaldehyde.
Analytical Biochemistry 101, 61–70.

incorporation of a co-extruded plant protein (rapeseed and peas) on growth, nutrient utilization and muscle fatty acid composition of rainbow trout (Oncorhynchus mykiss). Aquaculture 113, 339–353.


Leatherland JF, Hilton JW & Slinger SJ (1987) Effects of thyroid hormone supplementation of canola meal-based diets on growth, and interrenal and thyroid gland physiology of rainbow trout (Salmo gairdneri). Fish Physiology and Biochemistry 3, 73–82.


© Nutrition Society 2000