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Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*)

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Abstract

Apparent digestibility coefficients (ADC) of nutrients and energy of extruded peas, extruded lupin and rapeseed meals were determined in juvenile rainbow trout and turbot. Extruded lupin was found to be a promising substitute for fish meal in the diets of trout and turbot, with an acceptable digestibility of its dry matter (70% in trout and 81% in turbot) and a high digestibility of its protein (96% in trout and 98% in turbot) and its energy (77% in trout and 85% in turbot). Extruded peas had a lower digestibility of its protein in trout (88%) than in turbot (92%), and the ADC of energy, mainly supplied as starch, was relatively low (69% in trout and 78% in turbot). The digestibility of rapeseed meal was improved by a thermal treatment. Without thermal treatment, rapeseed meal had a low digestibility of its dry matter (57%) and energy (69%) in turbot. The availability of phosphorus was higher for extruded lupin (62% in trout and 100% in turbot) compared to the other plant-ingredients. When compared to a solvent-extracted meal, the availability of phosphorus from rapeseed meal was improved by heat treatment in both species (42% vs. 26% in trout and 65% vs. 49% in turbot).

Keywords: Nutrition; Digestibility; *Oncorhynchus mykiss*; *Psetta maxima*; Peas; Lupin; Rapeseed; Phosphorus

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1. Introduction

Partial or even total replacement of dietary fish meal with soybeans (meal, extruded product or protein concentrate) has been successfully accomplished in a number of teleost fishes (Tacon, 1994; Kaushik et al., 1995; Mambrini et al., 1999). Given that plant ingredients in general have phosphorus contents lower than that of fish meals, the incorporation of soybean products in fish feeds is known to reduce phosphorus loads in the effluent water (Bergheim and Sveir, 1995; Cain and Garling, 1995). However, since European production of soybean is extremely limited, it is considered worth exploring the possibilities of using other ingredients (pulses, cereals or oil seeds) as fish meal substitutes.

While the content of protein of peas is low ($< 25\%$ dry matter, DM), it is rich in starch ($> 50\%$ DM), with a relatively high energy content. In species such as salmonids, the digestive utilisation of polysaccharides or polyholosides is limited (Singh and Nose, 1967; Palmer and Ryman, 1972), but heat treatments, such as extrusion, are known to break the structure of starch, and thus, greatly improve its digestibility (Bergot, 1979; Bergot and Brèque, 1983; Hilton et al., 1981; Kaushik 1989). Extruded peas have been successfully used as a fish feed ingredient (Gomes and Kaushik, 1989; Gomes et al., 1993; Gouveia et al., 1993). Dehulling leads to a decrease in the fibre content of pea seeds to improve their digestibility. Thanks to genetic selection, current pea varieties contain no tannins but only low levels of trypsin inhibitors and lectins (Bond and Duc, 1993), which can be further deactivated with the high temperatures occurring in the extrusion process (Melcion and van der Poel, 1993). Many improvements have been made in the processing conditions of extrusion, preventing excessive heating and Maillard reaction, which both decreases protein digestibility.

Several works have also demonstrated the interest of the incorporation of lupin seed meals, especially dehulled and extruded lupin, in the diets for rainbow trout (De la Higuera et al., 1988; Gomes and Kaushik, 1989; Hughes, 1988; Moyano et al., 1992; Bangoula et al., 1993; Gouveia et al., 1993). Lupin seeds have a high content of protein (35–50% DM) and are relatively rich in oil (about 10%), and strains of white lupin (*Lupinus albus*) with low alkaloid contents are now available (Roemer, 1993). But lupin also contain high proportions of α-galactosides (7–15% DM) and fibre (25–30% DM) (Ferrando and Blum, 1989), reducing the digestibility of its dry matter. Dehulling indeed decreases the fibre content (Melcion and van der Poel, 1993), whereas it has no effect on α-galactosides, mainly localised in cotyledons. But extrusion treatment increases the utilisation of the components of lupin (Bangoula et al., 1993), and especially, of the nitrogen-free extracts.

Rapeseed is mainly an oil-source (40–45% DM), but the rapeseed meal obtained after extraction of oil is an interesting protein-source with protein content varying between 32% and 45% DM. In Europe, it is *Brassica napus oleifera* that is mainly cultivated. Rapeseed meal has been the object of numerous studies in rainbow trout as potential substitute to fish meal (Yurkowski et al., 1978; Hilton and Slinger, 1986; Gomes and Kaushik, 1989; Gomes et al., 1993; Abdou Dade et al., 1990; McCurdy and March, 1992). While the advantage of rapeseed meal is the quality of its protein (its amino acid profile is more interesting than that of soybean), it contains a high proportion of fibre

(30–40%) besides other anti-nutritional factors (ANF) such as tannins, sinapin and phytic acid. Rapeseeds are also known to contain glucosinolates (GLS), whose metabolites have a goitrogenic activity in all animals, including fish (Yurkowski et al., 1978; Higgs et al., 1982, Hardy and Sullivan 1983; Leatherland et al., 1987; Hossain and Jauncey, 1988; Teskeredzic et al., 1995). However, the quality of rapeseed meal has been considerably improved over the recent past with genetic selection of new varieties called ''colza 00'' with no erucic acid and with low GLS contents ($< 20–50$ μmol/g DM). Besides, technological treatments, such as dehulling and the utilisation of high temperatures and organic solvents during oil extraction, allow the decrease of the content of GLS, fibre, sinapin and tannins (Mawson et al., 1995; Higgs et al., 1996). The beneficial effects of dry extrusion for reducing the ANF content has also been shown (Fenwick et al., 1986; Smithard and Eyre, 1986), but such treatment is not yet currently applied on a regular basis.

The present study was undertaken to determine the digestibility of nutrients and energy of extruded peas, extruded and dehulled lupin, dehulled and solvent-extracted rapeseed meal and dehulled and heat-treated rapeseed meal (pressure-cooking) in freshwater-grown rainbow trout and seawater-grown turbot. The pea seeds used were not dehulled in order to establish if this treatment might be shunted for decreasing the cost of the technical treatment. The potential of solvent-extraction of rapeseeds, without heat treatment, was also tested for the ''colza 00'' owing to its economic advantage in comparison to that of the pressure-cooking currently used for common rapeseed meals.

2. Material and methods

2.1. Characteristics of plant-protein sources tested

Pea (*Pisum sativum*) and lupin (*L. albus*) seeds were provided by Union Nationale Interprofessionelle des Plantes Riches en Protéines (UNIP), Paris, France, and processed by Centre Technique Interprofessionel des Oléagineux Métropolitains (CETIOM), Bordeaux, France. After crushing (roller grinder; first stage diameter: 1.9 mm, second stage diameter: 1.2 mm), the rough flour obtained from peas was directly extruded (France extrusion; two long single-screw elements and two twin-screw elements; mean power: 27 kW; inlet moisture: 17%; temperatures: 186°C, 150°C and 102°C, for sleeves 1, 2 and 3, respectively; pressure: 26 bar; outlet rate: 158 kg/h; speed: 527 rpm; specific mechanical energy: 114 W h/kg). Crushed lupin seeds were dehulled by exhaustion (cleaner-separating system Denis D50) before being extruded (mean power: 18 kW; inlet moisture: 17%; temperatures: 128°C, 106°C and 92°C, for sleeves 1, 2 and 3, respectively; pressure: 38 bar; output rate: 101 kg/h; speed: 591 rpm; specific mechanical energy: 90 W h/kg).

Rapeseeds (*B. napus*), provided by CETIOM, were submitted to a very extensive dehulling treatment (seeds were blown towards a target by a propeller disk, then the hulls were separated from the kernels by air classification and exhaustion using a sorter with blowing bottom, and finally, kernels were sorted by exhaustion using a sieve on a cleaner-separating system (Denis D50), in order to reduce their fibre content). Then, two

different methods of fat extraction were used. The first method consisted of a direct oil extraction without heat treatment. Kernels were submitted to double-pressing under rollers, then the oil was extracted in a shaking filter (Guedu type ML 750) by eight consecutive hexane washings in percolation (partial filling of the filter with hexane, brewing during 15 min, then filtration over nitrogen pressure; temperature 35–45°C). The desolventation was realised by steam injection (3 bar, 5 min; maximal temperature: 80°C; total duration: 2 h). The second method for extracting oil, following a double-pressing of the kernels, consisted of a pressure-cooking (mean temperature: 97°C; pressure: 50 bar; output rate: 250 kg/h; mean duration: 1.5 h) with water injection (from 15 to 30 l/h) using a modern system of mechanical cooking and a power press. The residual oil was extracted by six consecutive hexane washings in percolation (temperature 30–35°C), followed by desolventation by steam injection (5 min; 6 bar; maximal temperature: 105°C; total duration: 74 min). Subsequent grinding produced the two rapeseed meals: solvent-extracted rapeseed meal and heat-treated rapeseed meal.

All ingredients were ground (≤ 400 μm) before incorporation into the diets. The chemical compositions of the ingredients tested are shown in Table 1.

2.2. Digestibility measurements

The apparent digestibility coefficients (ADC) for dry matter, protein, starch, total phosphorus and energy of the plant-protein sources were measured indirectly using

Table 1
Chemical composition of the ingredients tested on a dry matter basis

	Fish meal[a]	Extruded peas	Extruded lupin	Rapeseed meal solvent extracted	Rapeseed meal heat treated
Dry matter (%)	89.7	90.9	92.8	93.7	91.5
Ash (%)	17.0	3.3	4.6	7.9	8.2
Crude protein (%)	70.9	26.0	43.4	43.1	43.3
Crude fat (%)	11.0	0.45	10.0	4.8	0.9
Starch (%)	–	51.7	–	–	–
Gross energy (kJ/g)	21.0	18.5	21.3	20.3	19.2
Total phosphorus (%)	2.46	0.44	0.54	1.49	1.56
Phytates (%)	–	0.28	0.38	1.17	1.25
Phytic acid (%)	–	0.97	1.34	4.15	4.43
Glucosinolates (μmol/g)	–	–	–	40	26
Rest (%)[b]	–	18.6	42.0	44.2	47.6
ADF (%)[c]	–	8.7	8.1	12.4	12.4
NDF (%)[c]	–	14.0	13.4	20.3	20.3
α-Galactosides (%)[c]	–	3.4	10.3	1.5	1.5

[a] High quality Norwegian herring meal treated at low temperature.

[b] Rest, theoretically corresponding to lignin, monosaccharides, non-starch polysaccharides and oligosaccharides was estimated as follows: 100 − (moisture + ash + crude protein + crude fat + starch).

[c] The values indicated for ADF, NDF and α-galactosides are the averages obtained from about 20 samples of similar plant-ingredients (UNIP and CETIOM, personal communication).

chromic oxide as an inert tracer by the method of substitution (Sugiura et al., 1998; Bureau et al. 1999). Two reference diets were prepared for trout and turbot, respectively, containing 1% chromic oxide. The test diets were made by mixing 70% of the reference diet mixture and 30% of the respective plant-ingredient to be tested as shown in Table 2. Pellets (∅ 4 mm) were prepared using a power press Simon-Hensen (Simon-Hensen, Holland; dry pelleting). In all the measurements, fish were fed by hand to the reference or the test diets to apparent satiation in one daily meal.

The digestibility measurements in rainbow trout (*Oncorhynchus mykiss*) were carried out in the INRA facilities at St Pée-sur-Nivelle (France) using 10 cylindro-conical tanks (capacity: 60 l; water-flow rate: 5 l/min) supplied with recycled freshwater (temperature 16.5°C). Twenty rainbow trout (about 100 g each) were randomly allocated to each tank. Fish were submitted to a 1-week adaptation period to the experimental conditions (12 h of light:12 h of dark) and experimental diets. Then, faecal samples were collected for 1

Table 2
Ingredients and chemical composition of diets used in the digestibility measurements

Diets	Rainbow trout					Turbot				
	Ref. 1	Test 1	Test 2	Test 3	Test 4	Ref. 2	Test 1	Test 2	Test 3	Test 4
Ingredients (%)										
Extruded peas		30					30			
Extruded lupin			30					30		
Rapeseed meal, solvent-extracted				30					30	
Rapeseed meal, heat-treated					30					30
Fish meal	65	46.9	46.9	46.9	46.9	75	52.5	52.5	52.5	52.5
Gelatinised wheat starch	17	11.9	11.9	11.9	11.9	8	5.6	5.6	5.6	5.6
Fish oil	12	8.4	8.4	8.4	8.4	10	7	7	7	7
Vitamin mixture[a]	2	1.4	1.4	1.4	1.4	2	1.4	1.4	1.4	1.4
Mineral mixture[b]	2	1.4	1.4	1.4	1.4	2	1.4	1.4	1.4	1.4
Sodium alginate	1	0.7	0.7	0.7	0.7	2	1.4	1.4	1.4	1.4
Chromic oxide	1	0.7	0.7	0.7	0.7	1	0.7	0.7	0.7	0.7
Chemical composition										
Dry matter (%)	89.5	91.6	91.5	87.4	87.6	88.9	88.2	86.9	87.1	86.7
Crude protein (% DM)	45.5	40.3	46.6	46.2	46.8	54.3	47.1	52.3	52.4	52.2
Starch (% DM)	14.4	22.8	10.5	8.6	9	6.4	16.2	5.8	4.4	4.1
Gross energy (kJ/g DM)	22.1	21.1	21.8	21.2	21.3	21.6	21.0	21.7	21.1	21.2
Phosphorus (% DM)	1.84	1.44	1.42	1.68	1.75	2.05	1.53	1.60	1.84	1.86

[a]As per National Research Council (NRC) (1993).

[b]In g or mg/kg diet: calcium carbonate (40% Ca), 1.12 g; magnesium oxide (60% Mg), 0.62 g; ferric citrate, 0.1 g; potassium iodide (75% I), 0.2 mg; zinc sulphate (36% Zn), 0.2 g; copper sulphate (25% Cu), 0.15 g; manganese sulphate (33% Mn), 0.15 g; dibasic calcium phosphate (20% Ca, 18% P), 2.5 g; cobalt sulphate, 1 mg; sodium selenite (30% Se), 1.5 mg; KCl, 0.45g; NaCl, 0.2 g.

week from duplicate groups of fish per dietary treatment, using an automatic faecal collector (Choubert et al., 1982) and the collected faecal samples were frozen daily.

The measurements in turbot (*Psetta maxima*) were carried out in the IFREMER facilities at Brest (France), using 10 cylindro-conical tanks (capacity: 500 l; water-flow rate: 5 l/min) supplied with fresh thermo-regulated seawater (salinity: 35‰, 16°C). Thirty-five turbots (about 110 g each) were randomly allocated to each tank. Following a 2-week period of adaptation to rearing conditions (12 h of light:12 h of dark) and experimental diets, faecal samples were collected from the two groups of fish per dietary treatment over 1 week, using the faeces settling column system similar to the one described in Cho et al. (1985) but adapted to cylindro-conical tanks. Samples, constituted of faeces and residual water, were centrifuged in order to recover the nitrogen compounds in suspension, and kept frozen. The faeces of turbot have a tendency to loose their consistency in the water-column. In order to prevent this disintegration, a higher proportion of binder (alginate) was added in the turbot diets compared to the trout diets (see Table 2).

Pooled faeces from each group of fish were freeze-dried prior to the analysis for chromic oxide, protein, fat, energy, ash and phosphorus content. ADCs of the reference diet were calculated according to Maynard and Loosly (1969):

$$\text{ADC of dry matter of diet (\%)} = 100 \times [1 - (\text{dietary } Cr_2O_3/\text{faecal } Cr_2O_3)]$$

$$\text{ADC of nutrients and energy of diet (\%)} = 100 \times [1 - (\text{dietary } Cr_2O_3/\text{faecal } Cr_2O_3) \times (\text{faecal nutrient or energy concentration}/\text{dietary nutrient or energy concentration})]$$

The ADC of dry matter, nutrients and energy in the tested ingredients were calculated as follows (Sugiura et al., 1998):

$$\text{ADC of dry matter of test ingredient (\%)} = (\text{ADC of the test diet} - 0.7 \times \text{ADC of the reference diet})/0.3$$

$$\text{ADC of nutrient or energy of test ingredient (\%)} = [(\text{nutrient or energy concentration in test diet} \times \text{nutrient or energy ADC of the test diet}) - (0.7 \times \text{nutrient or energy concentration in reference diet} \times \text{nutrient or energy ADC of the reference diet})]/(0.3 \times \text{nutrient or energy concentration in ingredient})$$

All values are expressed per unit of dry matter. As ingredients were weighed and mixed with the reference diet on as fed basis (30% of the plant-protein source and 70% of the reference diet), the ratio 3:7 was corrected for the calculation for each test diet taking into account the dry matter contents of the test ingredient and of the reference diet.

2.3. Analytical methods

Analyses of ingredients, diets, and freeze-dried faecal samples were made following the usual procedures (AOAC, 1995): dry matter after drying at 105°C for 24 h; ash by

combustion at 550°C for 12 h; protein ($N \times 6.25$) by the Kjeldahl method after acid digestion; gross energy in an adiabatic bomb calorimeter (Parr Instruments, Moline IL); fat after extraction with petroleum ether by the Soxhlet method. Starch was measured by an enzymatic method (Thivend et al., 1972) using glucoamylase and glucose oxidase. Chromic oxide in the diet and faeces was determined according to Bolin et al. (1952). Total phosphorus was measured by spectrophotometric analysis of the phosphovanado-molybdate complex after mineralization and acid digestion (AFNOR, 1980). Phytic acid and phytate contents were determined according to Davies and Reid (1979).

2.4. Data analysis

Data are presented as means with standard error. To test the effect of the ingredients for each species, data were subjected to a one-way analysis of variance ($P < 0.05$), and when appropriate, means were compared by the Tukey's multiple range test or by the Student's *t*-test.

3. Results

The ADC values obtained with the two reference diets for protein and starch are from fish meal and gelatinised wheat starch, respectively, which are the main components of these reference diets. Hence, these values can be compared with those obtained from the plant-ingredients tested (Table 3).

Table 3
ADC (%) of the nutrients and energy of plant-ingredients in rainbow trout and turbot, and comparison, when possible, with those of fish meal and gelatinised wheat starch contained in the reference diet.

	Fish meal	Gelatinised starch	Extruded peas	Extruded lupin	Rapeseed meal, solvent-extracted	Rapeseed meal, heat-treated	
Trout							
Dry matter			66.3 ± 3.9	69.7 ± 1.7	70.8 ± 3.5	66.6 ± 3.1	NS
Protein	89.2 ± 0.2^{a}		87.9 ± 0.2^{a}	96.2 ± 0.7^{b}	90.9 ± 2.3^{a}	88.5 ± 1.5^{a}	$P < 0.01$
Energy			68.9 ± 3.2^{a}	77.0 ± 1.3^{b}	76.4 ± 2.4^{b}	70.0 ± 3.1^{ab}	$P < 0.05$
Starch		98.3 ± 0.1^{b}	82.8 ± 4.8^{a}				$P < 0.05$
Phosphorus			42.6 ± 1.4^{b}	61.9 ± 3.3^{a}	26.4 ± 7.5^{a}	41.8 ± 2.6^{b}	$P < 0.001$
Turbot							
Dry matter			71.5 ± 4.8^{bc}	80.5 ± 0.3^{c}	57.1 ± 5.2^{a}	64.6 ± 4.9^{ab}	$P < 0.05$
Protein	90.8 ± 0.1^{b}		92.9 ± 4.8^{b}	97.8 ± 0.3^{b}	82.9 ± 4.9^{a}	91.9 ± 3.4^{b}	$P < 0.05$
Energy			77.7 ± 2.5^{b}	85.1 ± 1.0^{c}	69.3 ± 4.0^{a}	80.9 ± 0.9^{bc}	$P < 0.05$
Starch		81.6 ± 4.8	75.3 ± 2.5				NS
Phosphorus			$> 100^{c}$	$> 100^{c}$	49.3 ± 0.5^{a}	64.7 ± 4.8^{b}	$P < 0.001$

ADC values are given as means ($n = 2$) with standard error. Superscript letters indicate statistical differences (one way ANOVA and Tukey's multiple range test or *t*-test) between ADC values, related to the ingredients tested for each species. Means with no common letter are significantly different ($P < 0.05$).

In turbot, the digestibility of dry matter was significantly lower for the two rapeseed meals, especially the solvent-extracted one, compared to the extruded peas and extruded lupin. No significant difference was observed in rainbow trout.

In rainbow trout, the ADC of protein from all the plant-ingredients was comparable to that from fish meal, and even significantly higher in the case of extruded lupin. In turbot, the highest ADC values of protein were also recorded for extruded lupin, but values were not significantly different than those of extruded peas, heat-treated rapeseed meal and fish meal. The ADC of proteins was significantly lower for the solvent-extracted rapeseed meal.

The digestibility of the energy of the extruded peas was significantly lower than that of the extruded lupin and solvent-extracted rapeseed meal in rainbow trout. In turbot, the ADC values of energy of the solvent-extracted rapeseed meal were significantly lower than those of the other plant-ingredients. Digestible energy of extruded peas was also low in turbot, but differences were only significant when compared to that of extruded lupin. The digestibility of pea starch was significantly lower than that of gelatinised wheat starch in trout.

In rainbow trout, the digestibility of phosphorus was significantly higher for extruded lupin compared to that of all the other plant-ingredients. The lowest digestibility of phosphorus was observed for the solvent-extracted rapeseed meal. In turbot, the values were significantly higher for extruded lupin and extruded peas than those of other ingredients. The digestibility of phosphorus of the solvent-extracted rapeseed meal was also lower than that of the heat-treated rapeseed meal.

4. Discussion

In some cases, a high variability was observed between the ADC values obtained for the two groups of fish ($n = 2$) used per dietary treatment (coefficients of variation, CV, up to 19.3%). As already suggested by De la Noue et al. (1980), a greater number of replicates would have provided a higher statistical power for data analyses. Besides, given that two different methods of faecal collection were used, it is also not reasonable to draw conclusions as to whether the differences among ADC values for trout and turbot were species-related.

Protein from extruded peas, extruded lupin and heat-treated rapeseed meal appeared to be digested by rainbow trout and turbot as efficiently as those of a high quality Norwegian fish meal, in good agreement with the data of Sullivan and Reigh (1995) in hybrid striped bass. ADC of protein was high for these plant-ingredients in both species, and even significantly higher than that of the fish meal in the case of extruded lupin in trout, in accordance with the data of Hughes (1988) and Bangoula et al. (1993). ADC of protein from solvent-extracted rapeseed meal had a digestibility similar to that of the fish meal in trout, but in turbot, their digestibility was significantly lower. This would suggest that turbot are more sensitive to ANFs contained in rapeseed meal, although the activity of these factors was reduced by the thermal treatment. The levels of GLS were 26 and 40 μmol/g in heat-treated and solvent-extracted rapeseed meal, respectively. Indeed, a significant decrease of the digestibility of protein is observed in rat fed diets

containing progoitrin, epiprogoitrin or gluconapin (Bille et al., 1983; Bjerg et al., 1989), all present in rapeseed, but in smaller amounts, following a thermal treatment. The digestibility of protein has also been found to be lower in common carp when isothiocyanates (hydrolytic products of GLS) were added to the feed (Hossain and Jauncey, 1988).

The digestibility of starch contained in extruded peas was lower (83% in trout and 75% in turbot) than that of gelatinised wheat starch in both species, although it was not significant in turbot. These results suggest a lower level of gelatinisation in the starch of extruded peas. Digestibility of starch is also known to decrease with increasing dietary starch levels in trout (Bergot, 1979). In our case, the reference diets contained 17% and 8% of gelatinised wheat starch, and the diets containing peas contained 27% and 21% of starch from peas, for trout and turbot, respectively. It might be advisable to express the digestibility of one type of starch for a given level of dietary incorporation. Our results are, nevertheless, in good agreement with the data of Jollivet et al. (1988) reporting high ADC values (75–83%) for cooked cornstarch in turbot, with values comparable to those of Bergot and Brèque (1983) in trout.

In trout, there was no significant difference in the digestibility of dry matter among the plant-ingredients, due likely to the low statistical power of the experiment. The digestibility of the energy from peas was significantly lower than that of the extruded lupin and solvent-extracted rapeseed meal. This may be related to the fact that energy from peas is mainly from starch, which is generally less digestible than protein or fat. In addition, pea seeds were not dehulled and our own unpublished results have shown that dehulling improves the digestibility of dry matter and energy of this foodstuff. In turbot, the significant differences were found between the plant-ingredients in the ADC of dry matter. The digestibility of energy for this species did not follow the same pattern: the major difference was that the digestibility of the energy from extruded peas was lower than that for the heat-treated rapeseed meal. The imperfect parallelism between the ADC values of dry matter and those of energy is attributable to the different gross energy contents of carbohydrate and protein; as mentioned above, energy is mainly obtained from carbohydrate (starch and other saccharides) in extruded peas, instead of protein as in rapeseed meals and lupin.

The low digestibility of dry matter and energy of the solvent-extracted rapeseed meal found in turbot was probably due to the high fibre (acid detergent fibres (ADF) and neutral detergent fibres (NDF)) content of rapeseed meal (Table 1), as also shown by Hilton and Slinger (1986). Bangoula et al. (1993) reported that the extrusion of lupin improved the utilisation of the N-free extracts in rainbow trout. These authors suggested that this improvement was related to a higher breakdown of the cell walls, allowing a better access of the digestive enzyme to the cell components, and/or to a partial degradation of α-galactosides. Following extrusion at high temperature, a decrease of 30% of the concentration of these compounds was observed by Melcion (1987). Heat treatment seems to also have a beneficial effect on the utilisation of dry matter and energy in rapeseed meal. Indeed, the ADC values obtained with the heat-treated meal were higher than those obtained with the solvent-extracted meal. In addition, Gomes et al. (1995) found high digestibility values in trout for a co-extruded product of a mixture of rapeseed and peas. However, an effect of GLS contained in higher proportion in the

solvent-extracted rapeseed should also be considered in turbot. Hossain and Jauncey (1988) found a decrease in dry matter digestibility in common carp when fed a feed supplemented with isothiocyanates. In trout, no significant difference was found among the two rapeseed meals for the digestibility of dry matter and energy. The ADC values obtained in this species were much higher than those reported by Higgs et al. (1996), but similar to those found by Hilton and Slinger (1986) and Abdou Dade et al. (1990).

The ADC values of phosphorus from plant-ingredients, especially extruded peas and lupin, were clearly over-estimated in turbot. Literature reports a phosphorus availability of 19–58% for several fish species including rainbow trout for plant-products such as rice and soybean meal (Lall, 1991). This over-estimation may be caused by the leaching of this compound from faeces in the column before sampling. The leaching of phosphorus from faeces could induce a stronger over-estimation of its ADC values in the test diets containing peas and lupin than in the reference diet because of the very low concentration of this nutrient in these plant-ingredients. Secondly, the increase of the ADC values of this component in the test diets compared to the reference diet could be not only related to its higher availability in the 30% of peas or lupin, but also to an improvement of the digestibility of the phosphorus from the 70% of reference diet. The incorporation of plant-ingredients in the diet is reported to affect the intestinal transit-time, due to the presence of fibres and sugars (Storebakken et al., 1999). In addition, an increase of the absorption of phosphorus at the intestinal level, due to its lower concentration in the lupin or peas-based diets might be also an explanation.

Despite such an over-estimation of phosphorus availability in turbot, our data suggest that the available phosphorus was lower in the rapeseed meals, especially in the solvent-extracted meal, than in the extruded lupin in both fish species. Rapeseed meals used here had a higher level of phytic acid (4.15–4.43% DM), in tune with literature data (2.4–5.7% DM; Higgs et al., 1996), than lupin meal (1.3% DM). Phosphorus in the phytate form is known to be unavailable to fish, which have no endogenous or microbial phytase in their intestinal tract (Lall, 1991). Phytase is also present in plant seeds not submitted to thermal treatment, but its activity is low (< 200 IU/kg) in crude peas, lupin, or in rapeseed meal, compared with those of wheat or rye (up to 8000 IU/kg) (Pointillart, 1994). The lower ADC values of phosphorus from the solvent-extracted rapeseed meal compared to those of the heat-treated meal may be due to its higher GLS content. Hossain and Jauncey (1988) indeed showed a decline in ash digestibility with high isothiocyanate levels in the feed. Higher ADC values were observed for the ingredient (extruded lupin) having the lowest content of phytic acid and phytates, but also of total phosphorus. This argues in favour of the hypothesis that the lower the dietary concentration in phosphorus, the better is its digestibility, in agreement with the observations of Vielma and Lall (1998) in Atlantic salmon and of Breves and Schröder (1991) in terrestrial vertebrates. The results obtained with extruded peas are more difficult to explain because they were not in accordance with those of the extruded lupin in rainbow trout. How far the ADC of phosphorus is affected by dietary phosphorus level and by the phytic acid in fish remains to be investigated.

In conclusion, of the three plant protein sources tested here, extruded lupin appears to be the most promising substitute for fish meal in the diets of both trout and turbot. Extruded whole peas appear to have a lower digestibility of its protein and phosphorus

in trout, of its dry matter in turbot, and of its energy in both species. It might be advisable to remove the hulls of the pea seeds before extrusion in order to improve the digestibility of energy. Finally, the use of rapeseed meal, despite much progress in genetic engineering and processing technologies, appears limited. It is as yet difficult to point out which of the following factors, high fibre content, phytic acid or GLS, is individually or collectively involved. It might also be of interest to study the effect of extrusion on nutrient/energy digestibility of rapeseed meal.

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