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# Incorporation of Whole Lupin, *Lupinus albus*, Seed Meal in Commercial Extruded Diets for Rainbow Trout, *Oncorhynchus mykiss:* Effect on Growth Performance, Nutrient Digestibility, and Muscle Fatty Acid Composition

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## Abstract

**Whole lupin seed meal was evaluated as partial replacement for fishmeal in extruded diets for rainbow trout, with particular emphasis on the effect on growth performance and apparent digestibility coefficient (ADC) of protein, lipids, carbohydrates, and energy. Effect on muscle fatty acid composition was also evaluated. All diets were formulated to be isonitrogenous and isoenergetic and to contain approximately 45% crude protein and 5.5 kcal energy g/diet. Diets were formulated to include whole lupin seed meal at 0, 10, 15, and 20%. Triplicate groups of fish weighing 65.9 ± 15.1 g on average were fed twice a day until apparent satiation during 12 wk. Growth, feed intake, hepatosomatic index, hepatic histology, proximate composition of whole body, and muscular fatty acid profile were determined. Growth and feed utilization were similar in all treatments and whole-body composition did not vary among treatments. Polyunsaturated fatty acids of the n-6 and n-3 series remained constant in muscle as the amount of lupin in diets increased. Furthermore, the ADCs were similar among all diets. These results suggest that inclusion of whole lupin seed meal up to 20% in extruded diets for rainbow trout do not have any negative effect on growth, feed performance, or flesh quality.**

The accelerated growth of aquaculture has resulted in a high demand and use of marine protein sources for the formulation of feeds. Feed represents an important percentage of the operational costs in any intensive aquaculture process (Wurmann 2007). This elevated cost is mainly attributed to the use of fishmeal as the major protein source ingredient in the formulation of commercial feeds for most carnivorous aquatic species (Hardy and Barrows 2002). Currently, aquaculture uses an important proportion of the fishmeal world supply. In 2006, the aquaculture sector consumed about 3.06 million tonnes or 56.0% of world fishmeal production and 0.78 million tonnes or 87.0% of total fish oil production (Tacon and Nates 2007). Global availability of fishmeal has reached static production levels and the price has risen considerably (Barlow 2000; Davenport et al. 2003; FAO 2008). According to the projections, the demand for fishmeal in the coming years will increase significantly.

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For these reasons, rational use of marine protein sources and the formulation of nutritionally adequate aquafeeds, based on available and cost-effective alternative protein ingredients, are required in order to sustain the increasing development of aquaculture (Hasan 2001). Partial substitution of fishmeal using animal and plant alternative protein ingredients have been evaluated in diets for various cultured aquatic species with different results (Viyakarn et al. 1992; Pongmaneerat et al. 1993; Watanabe et al. 1993, 1997, 1998; Akiyama et al. 1995; Luzier et al. 1995; Riche and Brown 1999; Storebakken et al. 2000a; Satoh et al. 2003; Hernández et al. 2004).

Some of the main alternatives to be used as protein sources in fish feeds are plant ingredients, and many of them are widely available agro-industry by-products with very competitive prices (Tacon 1994; Aslaksen et al. 2007). The suitability of plant meals as dietary fishmeal substitutes in aquafeeds is highly dependent on the aquaculture species to be fed (Glencross et al. 2003; Glencross 2007). Salmonids as highly carnivorous fish need a high-quality (e.g. well balanced and highly digestible) protein source for optimum health and growth performance. Among potential plant raw materials, white lupin, with an elevated protein content (ca. 45) and a comparable energy content to fish meal, has been described as a feasible ingredient for the partial replacement of fishmeal in salmonid feeds. The substitution of fishmeal with lupin meal in diets for salmonid species has been reported with satisfactory results in terms of growth and digestibility by various authors (De la Higuera et al. 1988; Hughes 1988; Burel et al. 2000; Carter and Hauler 2000; Farhangi and Carter 2001; Bórquez and Alarcón 2002; Glencross et al. 2004; Glencross 2005). Chile has great potential for the production of grain legumes including lupin. This legume offers economic advantages over the use of other plant meals such as soybean, making it an attractive alternative source of protein in the national production of aquafeeds. Nevertheless, the potential to use different plant raw materials is related to the nutritional requirements of the species and should be properly studied (Guillaume and Metailler 1999). The use of legume seeds in most fish feeds is limited by the presence of endogenous antinutritional factors (ANFs) which accentuate digestive losses. Some ANFs are heat-labile and they are inactivated by heat treatment, which allows increased dietary levels at which such legumes can be utilized by fish (Tacon 1997; Fagbenro 1998). It is important that alternative plant feedstuffs present adequate bioavailability of nutrients, particularly digestible protein and energy. However, another important aspect to consider is the effect of the plant ingredient inclusion on the final quality of aquaculture products. The ingredient substitution could affect the fish by modifying the fatty acid composition of the feed (Storebakken et al. 2000b). An increase in the levels of n-6 polyunsaturated fatty acids (PUFAs) and a reduction in the levels of n-3 PUFAs in the tissues alter the nutritional value of the final product (Gomes et al. 1993; Torstensen et al. 2000; Bell et al. 2001; Caballero et al. 2002).

The present study was undertaken to evaluate the inclusion of whole lupin, *Lupinus albus,* var. Hamburgo, seed meal as a partial replacement for fishmeal in commercial extruded diets for rainbow trout, *Oncorhynchus mykiss*, with particular emphasis on the effect on growth performance, the apparent digestibility coefficient (ADC) (protein, lipids, carbohydrates, and energy), and muscle fatty acid composition.

## Materials and Methods

A feeding trial was conducted to determine the responses of rainbow trout to increasing dietary percentages of whole lupin seed meal in commercial extruded diets. All of the experimental diets were formulated to be isonitrogenous and isoenergetic and contained approximately 45% crude protein and 5.5 kcal g/diet. A fishmeal-based diet without whole lupin seed meal was used as positive control. Experimental diets were formulated to include whole lupin seed meal at 10, 15, and 20%. The formulations were calculated using the software Single-Mix® for Windows (FORMAT International Ltd, London, UK). The diets were

manufactured by Biomar Chile (Puerto Montt, Chile) using cooking extrusion technology. The formulation and chemical composition of the diets are shown in Table 1, while Table 2 shows the fatty acid profiles.

### *Fish, Rearing Conditions, and Sampling*

The experiment was carried out during 12 wk with rainbow trout at Los Laureles Experimental Fish Farm Station of the Universidad Católica de Temuco, Chile. A stock group of fish was fed with the control diet for a period of 10 d before the feeding experiment was initiated. Fish with an average body weight of about 65.9 ± 15.1 g were randomly assigned in triplicate groups to four dietary treatments (0, 10, 15, and 20% inclusion of whole lupin seed meal). Initially, the fish were distributed (46 fish/tank) at an average density of 10.7 kg/$m^3$ in twelve 0.3 $m^3$ circular fiber glass tanks. However, after 8 wk the fish were transferred to twelve 0.6 $m^3$ tanks because of the size increment achieved. The water supply was permanent and the influx rate corresponded to 7.5 and 15 L/min for the tanks of 0.3 and 0.6 $m^3$, respectively. Average water temperature was 13.5 ± 2.8 C during the whole experimental period. Fish were fed manually twice per day, 7 d a week to apparent satiation.

In order to determine growth changes and to calculate feed performance parameters, fish from each tank were individually weighed at the beginning and at the end of the experiment. Fifteen fish were randomly sampled from the original stock of fish for initial analysis of fatty acids profile, body composition, and liver histology. All groups of fish were starved during 24 h and anesthetized with benzocaine (BZ-20, Laboratorio Veterquimica, Chile) prior to weight measurement. At the end of the feeding trial, five fish per tank (15 per treatment) were randomly collected and euthanized. Body and liver weight of each fish were measured

TABLE 1. *Formulation and composition of the experimental diets.*

| | Experimental diets | | | |
|---|---|---|---|---|
| | Control | L10 | L15 | L20 |
| Ingredients (%) | | | | |
| Fishmeal[1] | 46.7 | 42.6 | 40.6 | 38.0 |
| Fish oil | 15.0 | 14.2 | 14.1 | 14.0 |
| Whole-grain lupin[2] | 0.0 | 10.0 | 15.0 | 20.0 |
| Wheat flour | 22.6 | 16.2 | 13.3 | 13.3 |
| Corn gluten[3] | 8.0 | 8.0 | 8.0 | 8.0 |
| Hydrolyzed feather meal[4] | 7.0 | 7.0 | 7.0 | 7.0 |
| Biomar premix | 0.7 | 0.7 | 0.7 | 0.7 |
| Water | 0.8 | 1.3 | 1.3 | 1.1 |
| Proximate composition (%)[5] | | | | |
| Dry matter | 92.50 | 95.61 | 92.36 | 91.73 |
| Protein | 45.07 | 46.96 | 46.42 | 45.36 |
| Lipid | 25.95 | 27.25 | 25.88 | 22.99 |
| Ash | 8.13 | 8.26 | 7.98 | 7.98 |
| Fiber | 0.74 | 1.48 | 2.22 | 2.05 |
| Nitrogen-free extract | 20.11 | 16.05 | 17.50 | 21.62 |
| Gross energy (kcal/g) | 5.62 | 5.58 | 5.62 | 5.47 |

[1]Super Prime Chilean fish meal, steam-dried (Exapesca S.A., Talcahuano, Chile): 68% crude protein and 12% crude lipid.

[2]White lupin var. Hamburg (Sel Chile S.A., Temuco, Chile): 37.3% crude protein, 7.4% crude lipid, and total alkaloid 0.012%.

[3]By-product from starch and corn oil industry (Graneles Chile S.A., Santiago, Chile): 60% crude protein and 3% crude lipid.

[4]By-product from poultry industry (Terramar Chile S.A., Santiago, Chile): 82% crude protein and 6% crude lipid.

[5]Data are given on a dry matter basis.

TABLE 2. *Fatty acid composition of the experimental diets.*[1]

| | Experimental diets | | | |
|---|---|---|---|---|
| | Control | L10 | L15 | L20 |
| % Total lipid | 25.32 ± 0.88 | 23.74 ± 0.86 | 23.63 ± 0.57 | 24.70 ± 0.39 |
| C14:0 | 8.04 ± 0.24 | 8.49 ± 2.16 | 6.78 ± 1.65 | 6.49 ± 0.26 |
| C15:0 | 0.90 ± 0.02 | 0.91 ± 0.17 | 0.72 ± 0.20 | 0.66 ± 0.01 |
| C16:0 | 20.80 ± 0.09 | 21.70 ± 2.70 | 19.24 ± 1.63 | 19.40 ± 0.04 |
| C17:0 | 0.97 ± 0.04 | 0.96 ± 0.01 | 1.06 ± 0.17 | 0.93 ± 0.03 |
| C18:0 | 4.21 ± 0.08 | 4.17 ± 0.16 | 4.20 ± 0.01 | 4.24 ± 0.07 |
| C21:0 | 2.58 ± 0.10 | 2.40 ± 0.21 | 2.42 ± 0.02 | 2.30 ± 0.01 |
| C23:0 | 0.73 ± 0.04 | 0.39 ± 0.55 | 0.70 ± 0.01 | 0.64 ± 0.01 |
| Total SAFA[2] | 38.30 ± 0.17 | 39.36 ± 4.07 | 35.82 ± 3.46 | 35.09 ±0.28 |
| C16:1 | 6.47 ± 0.04 | 6.45 ± 0.64 | 5.61 ± 0.30 | 5.57 ± 0.05 |
| C17:1 | 1.04 ± 0.07 | 1.09 ± 0.14 | 1.00 ± 0.00 | 1.04 ± 0.00 |
| C18:1n9 | 12.47 ± 0.05 | 12.85 ± 0.44 | 14.56 ± 0.36 | 15.33 ± 0.07 |
| C20:1n9 | 1.46 ± 0.05 | 1.41 ± 0.22 | 1.49 ± 0.20 | 1.49 ± 0.01 |
| C24:1n9 | 0.48 ± 0.00 | 0.42 ± 0.01 | 0.45 ± 0.02 | 0.45 ± 0.05 |
| Total MUFA[3] | 24.85 ± 0.03 | 25.00 ± 0.06 | 26.16 ± 0.26 | 26.94 ± 0.11 |
| C18:2n6t | 1.60 ± 0.02 | 1.65 ± 0.25 | 1.47 ± 0.08 | 1.46 ± 0.02 |
| C18:2n6c | 5.90 ± 0.12 | 6.19 ± 0.35 | 6.39 ± 0.00 | 6.76 ± 0.07 |
| C18:3n3 | 1.27 ± 0.06 | 1.36 ± 0.01 | 1.58 ± 0.06 | 1.65 ± 0.01 |
| C18:3n6 | 0.40 ± 0.01 | 0.37 ± 0.00 | 0.44 ± 0.02 | 0.42 ± 0.03 |
| C20:3n6 | 0.50 ± 0.04 | 0.44 ± 0.06 | 0.42 ± 0.05 | 0.42 ± 0.05 |
| C20:4n6 | 1.24 ± 0.02 | 1.23 ± 0.06 | 1.31 ± 0.05 | 1.33 ± 0.04 |
| C20:5n3 | 11.63 ± 0.09 | 10.77 ± 1.75 | 11.48 ± 0.93 | 10.91 ± 0.06 |
| C22:6n3 | 14.07 ± 0.44 | 13.50 ± 2.69 | 14.67 ± 2.30 | 14.34 ± 0.03 |
| Total PUFA[4] | 36.86 ± 0.20 | 35.64 ± 4.13 | 38.03 ± 3.20 | 37.96 ± 0.17 |
| $\sum$ n-3[5] | 26.97 ± 0.29 | 25.64 ± 4.43 | 27.73 ± 3.29 | 27.30 ± 0.05 |
| $\sum$ n-6[6] | 9.89 ± 0.09 | 10.00 ± 0.30 | 10.33 ± 0.10 | 10.66 ± 0.12 |
| $\sum$ n-3[1]/$\sum$ *n*-6[7] | 2.73 ± 0.06 | 2.57 ± 0.52 | 2.69 ± 0.34 | 2.56 ± 0.02 |
| $\sum$ n-3 HUFA[8] | 25.69 ± 0.35 | 24.28 ± 4.44 | 26.15 ± 3.23 | 25.64 ± 0.04 |
| C18:1n9/DHA[9] | 1.08 ± 0.04 | 1.17 ± 0.20 | 1.20 ± 0.15 | 1.27 ± 0.01 |

HUFA = highly unsaturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SAFA = saturated fatty acid.

[1]Each value is the average ± standard deviation ($n = 6$).

[2]Total saturated fatty acids, including fatty acids not listed.

[3]Total monounsaturated fatty acids, including fatty acids not listed.

[4]Total polyunsaturated fatty acids, including fatty acids not listed.

[5]Total omega-3 fatty acids, including fatty acids not listed.

[6]Total omega-6 fatty acids, including fatty acids not listed.

[7]Total omega-3: omega-6 ratio, including fatty acids not listed.

[8]Total omega-3 highly unsaturated fatty acids.

[9]Oleic acid : docosahexaenoic acid ratio.

and registered. Analyses of pooled whole body samples from each group of fish were made to determine protein, lipid, moisture, and ash. Pooled dorsal muscle samples (three fish per tank) from each treatment were collected and frozen at −20 C during 2 wk for fatty acid profile analysis.

Thermal growth coefficient (TGC) was determined by the equation $\mathrm{TGC} = (W_f^{1/3} - W_i^{1/3})/\sum(T \times D) \times 100$, where $W_i$ and $W_f$ are the initial and final weights (tank means), respectively, $D$ represents the number of feeding days, and $T$ corresponds to the average water temperature. Feed conversion ratio (FCR) was calculated as $\mathrm{FCR} = F/G$, where $F$ is consumption of dry matter from feed and $G$ is the weight gain. Hepatosomatic index (HSI) was calculated as $\mathrm{HSI} = 100 \times (\mathrm{LW}/\mathrm{FW})$, where LW is the liver weight and FW represents the fish's body weight.

ADCs were determined using an indirect quantification method with $Cr_2O_3$ as inert marker. Twelve tanks were stocked each with 65 fish (30.08 ± 9.95 g). Fish were acclimatized to the tanks and to each dietary treatment during 10 d before initiating the fecal collection. Fish were fed manually twice a day, and feces were collected using a settlement column fecal collector as described by Cho et al. (1985). Feces were collected over a 7-d period, pooled within the tank, and kept at −80 C before being freeze-dried for 48 h in preparation for analysis. Dry matter ADC (%) was calculated by means of the formula $100 \times [1 - (\%I_{\text{feed}}/\%I_{\text{feces}})]$ and ADC of the nutrients (%) as $100 - [100(\%I_{\text{feed}}/\%I_{\text{feces}}) \times (\%N_{\text{feed}}/\%N_{\text{feces}})]$, where $I$ is the inert marker and $N$ the nutrient. The digestible energy (DE) was calculated according to De la Higuera et al. (1988) as $\text{DE} = (F_{\text{protein}} \times \text{ADC}_{\text{protein}} \times E_{\text{protein}}) + (F_{\text{lipid}} \times \text{ADC}_{\text{lipid}} \times E_{\text{lipid}}) + (F_{\text{nitrogen-free extract}} \times \text{ADC}_{\text{nitrogen-freeextract}} \times E_{\text{nitrogen-free extract}})$, where $F$ is the nutrient content of the feed (g), ADC is the apparent digestibility of the nutrient, and $E$ is the theoretical energy content (kJ/g).

### *Chemical Analysis*

The proximate compositions (crude protein, crude lipid, total fiber, ash, and moisture) of diets, feces, and carcass were determined according to AOAC (1998). Dry matter was calculated by gravimetric analysis following oven-drying at 105 C for 24 h. Chromic oxide levels were determined spectrophotometrically following the digestion and oxidation of samples using a modification from Furukawa and Tsukahara (1966). Protein levels were calculated from the determination of total nitrogen by Kjeldahl digestion, based on N × 6.25. Fat content was determined gravimetrically following extraction of the lipids with solvent (Soxhlet). Ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace at 550 C for 3 h. Fiber content was calculated by gravimetric analysis following oven-drying at 105 C for 24 h, after acid and alkali digestion with sulfuric acid and sodium hydroxide, respectively. Nitrogen-free extract (NFE) content was determined by difference. The gross energy in diets was estimated assuming a theoretical energetic content of 5.6, 9.5, and 4.1 kcal/g for the proteins, lipids, and carbohydrates, respectively (Cho et al. 1982).

The total lipids of diet and dorsal muscle samples were extracted according to Folch et al. (1957), from a 1-g sample which was homogenized in a chloroform/methanol solution (2:1; v/v) and 0.01% butyl hydroxytoluene (BHT). Fatty acids methyl esters (FAME) were prepared by acid catalysis transmethylation of total lipids (Morrison and Smith 1964). FAME were analyzed using a Hewlett Packard gas chromatograph (model HP6890 GC System, Elisan, USA), equipped with an FID detector and a fused silica capillary column SP2398 (30 m, 0.25 mm id, 0.20 μm film thickness), using helium as carrier gas. The initial oven temperature was 60 C. One minute after injection of the sample the temperature was increased to 245 C at a rate of 4 C. This temperature was maintained for an additional 5 min. Fatty acids were identified by reference to the Supelco-37 standard, expressed as a percentage of the area, according to the total fatty acids identified.

### *Histological Analysis*

The liver samples were fixed in Bouin alcohol, dehydrated in ethanol, and immersed in paraffin. The cut sections (4–6 μm) were stained using haematoxylin and eosin, for observation under the microscope.

### *Statistical Analysis*

The results were analyzed using the programme StatMost version 3.0 for Windows (Dataxiom Software Inc., Los Angeles, CA, USA). Significant differences ($P < 0.05$) between dietary treatments were determined by one-way analysis of variance (ANOVA). Differences between means were determined by Tukey's test. Before the application of the ANOVA, the presumed homogeneity of variances was verified through the Bartlett test.

Arcsine transformation was applied to data expressed as percentages. The normality of the data was confirmed using the Kolmogorov–Smirnov test.

## Results

All experimental diets were well accepted by the fish during the 12 wk of the experiment. The survival rate was nearly 95% for all treatments. Table 3 shows the results obtained for growth and feeding parameters. Final weight ranged from 236 g (control) to 260 g (L20), and no significant differences in growth occurred among treatments ($P > 0.05$).

No differences were observed in daily feed consumption. FCR was similar for all the treatments. TGC ranged between 0.17 and 0.19, and values were not significantly different ($P > 0.05$) among the dietary treatments.

The initial and final body compositions and HSI of the fish are shown in Table 4. In general, the inclusion of whole-grain lupin in the diets did not significantly affect the proximate composition of the fish compared to the control diet. Nevertheless, among the treatments with lupin inclusion, only protein content was different between L15 and L10 diets ($P < 0.05$). On the other hand, at the end of the growth trial, fish in all treatments exhibited a significant decrease in protein, dry matter, and ash content, and a significant increase in lipid content ($P < 0.05$) compared to the initial body composition. HSI did not differ between treatments, with values close to 1.0%. However, these values were significantly lower ($P < 0.05$) than those determined at the beginning of the experiment.

Liver histology of fish fed diets with lupin was similar to fish fed the control diet. Some lesions due to infiltration of fat in the hepatocytes were observed for all of the treatments compared to the initial state of the livers.

ADC values for all the experimental diets were not significantly different (Table 5). ADC

TABLE 3. *Growth and feeding parameters in rainbow trout fed with different experimental diets.*[1]

| | Experimental diets | | | |
|---|---|---|---|---|
| Parameter | Control | L10 | L15 | L20 |
| Initial weight (g) | 65.9 ± 15.1 | 65.9 ± 15.1 | 65.9 ± 15.1 | 65.9 ± 15.1 |
| Final weight(g) | 236.1 ± 56.1 | 255.7 ± 59.9 | 242.1 ± 67.2 | 260.5 ± 57.4 |
| % weight increase | 258.2 ± 15.4 | 281.3 ± 15.4 | 268.5 ± 28.1 | 295.3 ± 35.4 |
| Daily feed intake (g) | 98.7 ± 9.7 | 100.8 ± 4.9 | 105.0 ± 6.9 | 95.2 ± 1.2 |
| FCR[2] | 1.07 ± 0.12 | 1.0 ± 0.03 | 1.16 ± 0.04 | 1.02 ± 0.03 |
| TGC[3] | 0.17 ± 0.00 | 0.18 ± 0.01 | 0.17 ± 0.01 | 0.19 ± 0.02 |

[1]Each value is the average ± standard deviation ($n = 3$). None of the values was significantly different.
[2]Feed conversion ratio.
[3]Standard growth rate. Thermal growth coefficient.

TABLE 4. *Body composition and hepatosomatic index of rainbow trout,* Oncorhyncus mykiss*, fed with different experimental diets.*[1]

| Proximate composition (%) | Initial | Experimental diets | | | |
|---|---|---|---|---|---|
| | | Control | L10 | L15 | L20 |
| Dry matter | 28.29[a] | 23.77 ± 0.92[cb] | 25.37 ± 0.65[ac] | 21.27 ± 1.72[b] | 23.98 ± 0.98[cb] |
| Protein | 18.88[a] | 13.34 ± 0.81[bc] | 14.96 ± 1.58[b] | 11.40 ± 0.37[c] | 13.09 ± 0.71[bc] |
| Lipid | 5.99[a] | 8.66 ± 0.98[ab] | 10.27 ± 1.37[ab] | 10.59 ± 1.26[b] | 7.90 ± 0.29[b] |
| Ash | 2.66[a] | 1.63 ± 0.35[b] | 2.07 ± 0.11[ab] | 1.41 ± 0.27[b] | 1.99 ± 0.18[ab] |
| HSI[2] | 1.9 ± 0.32[a] | 1.1 ± 0.27[b] | 1.0 ± 0.18[b] | 1.1 ± 0.12[b] | 1.1 ± 0.23[b] |

[1]Each value is the average ± standard deviation ($n = 3$). Values in the same row with distinct superscripts are significantly different ($P < 0.05$). Data are given on a wet weight basis.
[2]Hepatosomatic index.

TABLE 5. *Apparent digestibility coefficients and digestible energy for the experimental diets.*[1]

| | Experimental diets | | | |
|---|---|---|---|---|
| | Control | L10 | L15 | L20 |
| Dry matter | 84.80 ± 1.83 | 83.90 ± 0.96 | 86.88 ± 1.77 | 86.46 ± 1.38 |
| Protein | 92.77 ± 0.65 | 92.23 ± 0.36 | 94.32 ± 0.74 | 94.35 ± 0.71 |
| Lipid | 98.93 ± 0.33 | 95.28 ± 0.33 | 97.29 ± 0.38 | 98.36 ± 0.32 |
| Nitrogen-free extract | 75.70 ± 3.07 | 70.06 ± 0.02 | 76.91 ± 2.50 | 78.83 ± 0.37 |
| Digestible energy (MJ/kg) | 22.66 ± 0.24 | 22.51 ± 0.08 | 22.74 ± 0.02 | 22.12 ± 0.12 |

[1]Each value is the average ± standard deviation ($n = 3$). Data are given in dry matter basis.

values for dry matter varied between 83.9% (L10) and 86.5% (L20). The highest ADC values were found for lipids, followed by protein, while the lowest coefficients were obtained for carbohydrates.

Dorsal muscle fatty acid compositions of fish fed experimental diets are reported in Table 6. The percentage of saturated fatty acids (SAFAs) was not affected by the incorporation of whole-grain lupin. The most abundant fatty acid was palmitic acid (C16:0). This fatty acid decreased with increasing levels of dietary lupin; however, this was not significant.

The total amount of monounsaturated fatty acids (MUFAs) had a slight tendency to increase, but there were no significant differences between the control diet and the diets with lupin. However, the initial MUFA content of the muscle was significantly increased in all treatments ($P < 0.05$) because of the significant increases in palmitoleic (C16:1) and elaidic (C18:1n-9 trans) acids.

The total PUFAs had a slight tendency to increase as the percentage of lupin in the feed increased, although this was not significant ($P > 0.05$). There was no difference for the different diets in the n-6 PUFA content of the muscle; however, the linoleic (C18:2n-6 cis) and arachidonic (C20:4n-6) acids decreased by the end of the experiment as compared to the initial levels ($P < 0.05$).

The percentage of linoleic acid (C18:3n-3) varied between 0.85% (L10) and 1.19% (L20), and the values for eicosapentaenoic acid (EPA, C20:5n-3) fluctuated between 6.08% (control) and 7.5% (L20).

The docosahexaenoic acid (DHA; C22:6n-3) in the muscle was constant between the treatments. These percentages were slightly greater than those present in the diets, although not significantly different ($P > 0.05$).

Despite the decrease in the total n-6 PUFAs, the n-3/n-6 ratio was not affected by the diets ($P > 0.05$). However, the C18:1n-9/DHA ratio in the muscle was similar ($P > 0.05$) between the dietary treatments.

## Discussion

This study demonstrated the potential for using whole-grain lupin in commercial rainbow trout feed, in agreement with the previously reported studies on salmonids (De la Higuera et al. 1988; Burel et al. 1998, 2000; Carter and Hauler 2000; Farhangi and Carter 2001; Glencross 2007). In general, the growth performances obtained in the fish fed diets with whole grain lupin, regardless of the percentage incorporated, were similar to those obtained with the control diet.

Lupin contains antinutritional factors, principally alkaloids (Tacon 1997; Aslaksen et al. 2007), which could affect the feed intake because of palatability issues (De la Higuera et al. 1988). However, in agreement with Morales et al. (1994), feed intake recorded in this experiment was not influenced by dietary lupin concentration. Further, the variety used in this study was a sweet type, with low levels of alkaloids, below 100 mg/kg (Petterson et al. 1997, 2000).

Although weight gain has been shown to decrease with increasing dietary lupin in the previous studies (De la Higuera et al. 1988; Hughes 1991), the low levels of dietary inclusion in the present experiment had no effect.

TABLE 6. *Profile of fatty acids present in the muscle of rainbow trout fed with different experimental diets.*[1]

| | | Experimental diets | | | |
|---|---|---|---|---|---|
| | Initial | Control | L10 | L15 | L20 |
| % Total lipid | 1.30 ± 0.36[a] | 2.25 ± 1.85[a] | 4.34 ± 0.78[a] | 4.66 ± 2.08[a] | 5.34 ± 4.07[a] |
| C14:0 | 3.03 ± 0.62[a] | 5.89 ± 0.42[b] | 6.41 ± 0.55[b] | 5.70 ± 0.49[b] | 5.43 ± 0.59[b] |
| C16:0 | 28.24 ± 5.00[a] | 28.13 ± 2.97[a] | 25.64 ± 1.19[ab] | 22.88 ± 0.99[ab] | 21.78 ± 2.29[b] |
| C17:0 | 0.00 ± 0.00[a] | 0.69 ± 0.10[b] | 0.84 ± 0.31[b] | 0.75 ± 0.44[b] | 0.58 ± 0.14[b] |
| C18:0 | 7.06 ± 0.91[a] | 6.41 ± 1.43[ab] | 4.98 ± 0.53[a] | 4.68 ± 0.28[b] | 4.47 ± 0.65[b] |
| C21:0 | 0.67 ± 0.15[a] | 0.89 ± 0.09[b] | 1.00 ± 0.05[b] | 1.21 ± 0.11[b] | 1.25 ± 0.13[b] |
| C23:0 | 0.00 ± 0.00[a] | 0.67 ± 0.20[b] | 0.82 ± 0.05[b] | 0.89 ± 0.10[b] | 0.95 ± 0.10[b] |
| Total SAFA[2] | 39.01 ± 6.68[a] | 44.00 ± 4.67[a] | 38.91 ± 2.09[a] | 37.31 ± 1.09[a] | 35.60 ± 2.70[a] |
| C16:1 | 3.62 ± 0.33[a] | 5.94 ± 0.64[b] | 6.19 ± 0.36[b] | 6.71 ± 0.47[b] | 6.33 ± 0.68[b] |
| C17:1 | 0.00 ± 0.00[a] | 0.80 ± 0.06[b] | 0.84 ± 0.05[b] | 0.93 ± 0.08[b] | 0.84 ± 0.15[b] |
| C18:1n9 | 14.12 ± 0.44[a] | 17.06 ± 0.78[b] | 16.95 ± 1.14[b] | 19.25 ± 1.14[bc] | 19.92 ± 1.02[c] |
| C20:1n9 | 0.89 ± 0.19[a] | 1.33 ± 0.68[a] | 1.55 ± 0.17[a] | 1.34 ± 0.67[a] | 1.85 ± 0.21[a] |
| C24:1n9 | 0.00 ± 0.00[a] | 0.40 ± 0.06[b] | 0.36 ± 0.21[b] | 0.37 ± 0.17[b] | 0.23 ± 0.24[b] |
| Total MUFA[3] | 21.45 ± 0.21[a] | 28.59 ± 1.64[b] | 29.20 ± 1.50[b] | 31.74 ± 1.83[b] | 32.69 ± 2.11[b] |
| C18:2n6t | 0.00 ± 0.00[a] | 0.80 ± 0.08[b] | 0.86 ± 0.08[b] | 0.73 ± 0.43[b] | 0.95 ± 0.17[b] |
| C18:2n6c | 6.65 ± 0.11[a] | 3.54 ± 0.26[bc] | 3.42 ± 0.18[b] | 3.85 ± 0.21[bc] | 4.10 ± 0.26[c] |
| C18:3n3 | 1.00 ± 0.01[a] | 0.91 ± 0.44[a] | 0.85 ± 0.04[a] | 1.19 ± 0.23[a] | 1.19 ± 0.10[a] |
| C18:3n6 | 0.00 ± 0.00[a] | 0.40 ± 0.22[a] | 0.23 ± 0.12[a] | 0.13 ± 0.15[a] | 0.16 ± 0.06[a] |
| C20:2 | 0.63 ± 0.21[a] | 0.63 ± 0.13[b] | 0.63 ± 0.16[ab] | 0.45 ± 0.16[ab] | 0.36 ± 0.07[ab] |
| C20:4n6 | 2.17 ± 0.93[a] | 1.05 ± 0.06[a] | 0.86 ± 0.38[a] | 1.12 ± 0.18[a] | 0.82 ± 0.35[a] |
| C20:5n3 | 6.73 ± 0.04[a] | 6.08 ± 1.01[a] | 6.68 ± 0.43[a] | 7.46 ± 0.28[a] | 7.46 ± 0.57[a] |
| C22:6n3 | 22.37 ± 7.69[a] | 13.43 ± 2.92[b] | 15.82 ± 1.52[ab] | 15.61 ± 1.62[ab] | 16.24 ± 1.64[ab] |
| Total PUFA[4] | 39.54 ± 6.89[a] | 27.41 ± 3.43[bc] | 29.91 ± 1.72[bc] | 30.95 ± 1.51[bc] | 31.71 ± 2.13[ac] |
| $\sum$ n-3[5] | 30.10 ± 7.72[a] | 20.62 ± 3.53[b] | 23.43 ± 1.81[b] | 24.32 ± 1.81[b] | 24.95 ± 1.90[b] |
| $\sum$ n-3 HUFA[6] | 29.10 ± 7.73[a] | 19.71 ± 3.84[b] | 22.58 ± 1.81[b] | 23.13 ± 1.72[b] | 23.76 ± 1.87[b] |
| $\sum$ n-6[7] | 9.44 ± 0.83 | 6.73 ± 0.17 | 6.48 ± 0.18 | 6.63 ± 0.64 | 6.76 ± 0.31 |
| $\sum$ n-3[1]/$\sum n-6$[8] | 3.24 ± 1.10[a] | 3.07 ± 0.53[a] | 3.62 ± 0.35[a] | 3.71 ± 0.60[a] | 3.69 ± 0.21[a] |
| C18:1n9/DHA[9] | 0.81 ± 0.28[a] | 1.53 ± 0.30[bc] | 1.27 ± 0.17[ac] | 1.44 ± 0.23[bc] | 1.44 ± 0.16[bc] |

HUFA = highly unsaturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SAFA = saturated fatty acid.

[1]Each value is the average ± standard deviation ($n = 6$).

[2]Total saturated fatty acids, including fatty acids not listed.

[3]Total monounsaturated fatty acids, including fatty acids not listed.

[4]Total polyunsaturated fatty acids, including fatty acids not listed.

[5]Total omega-3 fatty acids, including fatty acids not listed.

[6]Total omega-3 highly unsaturated fatty acids.

[7]Total omega-6 fatty acids including fatty acids not listed.

[8]Total omega-3 : omega-6 ratio, including fatty acids not listed.

[9]Oleic acid : docosahexaenoic acid ratio.

According to Farhangi and Carter (2001) and Burel et al. (2000), only inclusion of over 40% of *L. albus* in diets supplemented with amino acids caused a reduction in the weight gain in rainbow trout.

The TGC obtained in the experiment matched normal values reported for rainbow trout, which ranged between 0.153 and 0.203 (Bureau and Cho 1999). No effects on TGC have been demonstrated for lupin substitutions (Glencross et al. 2002), which suggests that the TGC values found were independent of the feeds tested, although they were lower than the values reported in the works cited.

Whole-body composition did not vary despite lupin inclusion, coinciding with the results of other studies where higher levels of lupine meal were included (Hughes 1991; Gouveia et al. 1993; Burel et al. 1998; Bórquez and Alarcón 2002; Carter and Hauler 2000; Bransden et al. 2001). However, the percentages of protein and lipid were lower than those achieved by these

studies, basically because of differences in fish sizes in each trial and whether the values are expressed in dry or wet basis.

The excess of lipids in the hepatic cells cannot be ascribed exclusively to the use of lupin because it was also observed in the control. The effect of the inclusion of legumes, such as lupin and peas, in the diet is characterized by an increase in lipid deposits and a reduction of glycogen in the hepatocytes (Robaina et al. 1995; Russell et al. 2001). Likewise, other factors such as a deficiency of highly unsaturated fatty acids (HUFAs) in the diet and a high density of fish in the tanks could provoke an increase in lipid deposits in the liver (Montero et al. 2001; Caballero et al. 2002). However, these alterations did not affect the HSI.

The ADC of dry matter, crude protein, and lipid found in this study were similar to those reported in salmonids (De la Higuera et al. 1988; Carter and Hauler 2000; Glencross et al. 2004) and turbot (Burel et al. 2000) when lupin meal was included in the extruded diets. Despite the fact that DE of legumes is considered low on account of their high carbohydrate content (Morales et al. 1994), the values of DE in the experiment were similar to those reported by Gomes et al. (1993) probably because of an improvement resulted by the extrusion process.

The fatty acid profile in rainbow trout muscle was directly related to the fatty acid profile of the respective feeds (Watanabe 1982; Bell et al. 1986, 2001; Gomes et al. 1993; Guillou et al. 1995; Henderson 1996; Montero et al. 2001; Caballero et al. 2002). When the amount of lupin was increased in the diets, a slight decrease in the total percentage of SAFA was noted in the muscle. This is particularly characteristic when low SAFA levels, plant ingredients-based feeds are offered because they cause a reduction of palmitic acid in the muscle (Gomes et al. 1993).

The high growth rate of farmed salmon is the result of the use of feeds rich in energy content, principally MUFAs, including oleic acid (Bell et al. 2001). The MUFA concentrations increase when lupin is used in the feed because plant proteins contain 18:1(n-9) as a major fatty acid, although this was not significant in the present experiment (Table 2), resulting in the accumulation of this fatty acid in the tissues of fish fed these protein sources (Gomes et al. 1993; Bell et al. 2001; Caballero et al. 2002); as observed in this work, fillet from fish fed diet L20 showed a significantly higher oleic acid content compared to fillet from control and L10, and however, not different from fish fed L15 (Table 6). This accumulation is a consequence of this species being unable to desaturate 18:1(n-9) into 18:3(n-3) or 18:2(n-6) (Storebakken et al. 2000b; Montero et al. 2001).

Another effect associated with the inclusion of plant protein sources is the increase in the n-6 PUFAs in the muscle, which is particularly high in lupin seeds (Yañez et al. 1983; Masson and Mella 1985; Petterson et al. 1997). Nevertheless, in the present study, with the inclusion of lupin in the diets, the 18:2n-6 levels in the muscle were similar to the control (Table 6), probably as a consequence of the minimum variation of n-6 fatty acid content in the experimental diets (Table 2). Similarly, the levels of 20:4n-6 did not vary among the treatments.

Lupin seed contains 9% linolenic acid (Yañez et al. 1983; Masson and Mella 1985; Petterson et al. 1997) and therefore its inclusion in the feeds could help to maintain a constant content of this fatty acid, as well as that of EPA and DHA. Adverse effects occur when using other plant ingredients which decrease the 18:3 n-3 in the tissues (Gomes et al. 1993; Guillou et al. 1995; Henderson 1996; Bell et al. 2001; Caballero et al. 2002).

The existence of a higher quantity of DHA in the muscle in comparison to that contained in the feed is in agreement with the studies conducted by Gomes et al. (1993) and Bell et al. (2001). According to these authors, this is a result of a selective accumulation of DHA in the muscle even though the amount in the feed is low. However, in the present trial, the DHA level in the muscles may be explained by the high fish meal and oil in all experimental diets.

The reduction in the initial percentages of n-6 and n-3 PUFAs may be due to the nutritional quality of the feed ingested by the fish before the experiment (Shearer 1994) or due to

the changes in the fatty acid profile produced by growth (Sheridan et al. 1985). Despite the fact that the quantity of lipids contributed by the lupin is small, the high proportion of n-6 series fatty acids causes an imbalance in the n-3/n-6 ratio (Robaina et al. 1995). However, this coefficient in the muscle is not affected by the diets because of the abundance of n-3 series PUFAs contributed by the fish oil, and in part by the lupin grain, and consequently our results were contrary to those reported with the use of other plant protein meals, where a reduction of the n-3/n-6 ratio is observed as a result of an increase in 18:2n-6 and a decrease in the percentage of n-3 PUFAs (Gomes et al. 1993).

The products of Chilean salmon farming are of high nutritional value, with an abundance of n-3 series HUFAs and a high n-3/n-6 PUFA ratio. The consumption of such products represents a valuable benefit to human health including the prevention of cardiovascular diseases (Corraze 1999). From a consumer viewpoint, it is essential that a high n-3/n-6 ratio is maintained in farmed fish. The use of plant proteins needs to be monitored carefully as these raw materials are known to influence the fatty acid profile of the final product (Bell et al. 2001). The use of fatty acid composition as a quality indicator of fish for human consumption shows that the inclusion of up to 20% of whole-grain white lupin in the extruded diets does not significantly alter the nutritional quality of the final product. On the other hand, although the inclusion of whole-grain white lupin in the extruded diets did not present technical or nutritional problems in the tested levels, further research should be carried out on the potential use of different species and varieties of lupin in aquafeeds for salmonids.

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