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Feeding high inclusion of whole grain white lupin (*Lupinus albus*) to rainbow trout (*Oncorhynchus mykiss*): effects on growth, nutrient digestibility, liver and intestine histology and muscle fatty acid composition

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Abstract

The effect of dietary inclusion of whole grain white lupin (*Lupinus albus*) on growth performance, histology, muscle fatty acid composition and nutrient digestibility was investigated in an 11-week growth and a 4-week digestibility trial with rainbow trout (initial body weight of 54.0 ± 6.2 and 181.9 ± 3.4 g respectively). Four experimental extruded diets were formulated to contain 0%, 30%, 40% and 50% of whole grain lupin and fed to triplicate groups of fish twice a day until apparent satiation. Faeces were collected daily from each digestibility tank by decantation. No significant trends were observed with respect to growth, feed utilization, apparent digestibility coefficients or whole-body composition ($P > 0.05$). Conversely, increasing levels of dietary lupin led to significant decreases in the Hepatosomatic index ($R^2 = 0.75$, $P < 0.05$) and slight lipid infiltration into hepatocytes and enterocytes. Muscle fatty acid compositions were slightly affected by the dietary treatment. Polynomial regression of dietary inclusion of lupin and muscle fatty acid concentrations showed an increase in C18:1n-9, C18:2n-6 and C18:3n-3 and a decrease in C20:5n-3 with increasing dietary lupin level. These results demonstrated that whole grain lupin can be included up to 50% in commercial rainbow trout diets without negative effects.

Keywords: lupin, growth performance, digestibility, fatty acid, histology

Introduction

Historically, salmonid diets have been formulated to contain fish meal as the most important source of dietary protein, comprising between 20% and 50% of the total ingredients (Watanabe 2002; Tacon & Metian 2008). The current production of fish meal is not sufficient to cover increasing demand from the aquaculture sector (Tveteras & Tveteras 2010). This has led to the partial replacement of fish meal with alternative sources of protein, primarily of plant origin such as legumes and oilseeds (Hardy 1996; Glencross, Booth & Allan 2007).

Lupin seeds have been used successfully as a replacement for fish meal in aquaculture feeds of salmonids and other marine fish (De la Higuera, Garcia-Gallego, Sanz, Cardenete, Suarez & Moyano 1988; Robaina, Izquierdo, Moyano, Socorro, Vergara, Montero & Fernandezpalacios 1995; Burel, Boujard, Corraze, Kaushik, Boeuf, Mol, Van der Geyten & Kuhn 1998; Burel, Boujard, Kaushik, Boeuf, Van der Geyten, Mol, Kuhn, Quinsac, Krouti & Ribaillier 2000; Carter & Hauler 2000; Farhangi & Carter 2001; Aslaksen,

Kraugerud, Penn, Svihus, Denstadli, Jorgensen, Hillestad, Krogdahl & Storebakken 2007; Glencross, Hawkins, Evans, Rutherford, Dods, McCafferty & Sipsas 2008). Incorporation of between 40% and 50% of lupin seed meal into diets for rainbow trout has been considered as the maximum level of inclusion in terms of growth and nutrient digestibility. Inclusion of lupin meal above this threshold can cause a drastic reduction in growth and increase lipid deposition (Burel *et al*. 1998; Farhangi & Carter 2001). Similar to other plant proteins, inclusion of high amounts of lupin seed in salmonids feeds can affect growth, feed intake and utilization of dietary nutrients due to the low content of lysine and methionine (Yanez, Ivanovic, Owen & Ballester 1983; Petterson, Sipsa & Mackintosh 1997), and also the presence of quinolizadine alkaloids and oligosaccharides (Francis, Makkar & Becker 2001; Krogdahl, Penn, Thorsen, Refstie & Bakke 2010).

To date, most of the research using lupins as a replacement for fish meal has been carried out with dehulled seed. Removal of the lupin seed coat from the seed kernel has compositional and nutritional benefits for experimental aquafeed (Glencross, Hawkins, Veitch, Dods, McCafferty & Hauler 2007). However, the practical application of this process under commercial conditions has yet to be demonstrated.

Whole grain lupin contains high levels of carbohydrates, mainly soluble and nonsoluble nonstarch polysaccharides and fibre (Daveby & Aman 1993). These components can cause negative changes in the chemical composition and nutritional value of the feed produced (Glencross, Booth *et al*. 2007). Excessive quantities of carbohydrates and nonstarch polysaccharides in fish diets have been reported to cause glycaemia, reduced digestibility, increased feed intake, decreased growth and faster gastric evacuation (Hilton, Atkinson & Slinger 1983; Francis *et al*. 2001; Hemre, Mommsen & Krogdahl 2002; Glencross, Boujard & Kaushik 2003; Glencross 2009).

On the other hand, as a consequence of the high inclusion of plant proteins, the chemical composition of the fish tissues can be affected considerably (De Francesco, Parisi, Medale, Lupi, Kaushik & Poli 2004).

The fatty acid compositions of the fish lipids are influenced by the fatty acid composition of dietary lipid and *de novo* synthesis of fatty acid in fish tissues (Watanabe 1982; Sargent, Bell, McEvoy, Tocher & Estevez 1999). Fish oil and fish meal are the main sources of fat in the diet for salmonids; these contain high levels of n-3 polyunsaturated fatty acids (PUFA) (Watanabe 2002). These fatty acids are required by salmonids in order to achieve normal growth and development (Watanabe 1982; Sargent *et al*. 1999).

Generally, the utilization of a high inclusion of plant protein in diets for salmonids has resulted in higher levels of oleic acid and linoleic acid, and lower levels of n-3 PUFA, linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in tissues of rainbow trout (Gomes, Corraze & Kaushik 1993; De Francesco *et al*. 2004; Morris, Gallimore, Handley, Hide, Haughton & Black 2005). This can cause changes in the nutritive value of the fish and influence fish physiology, as a result of the enzymatic competition among 18-C unsaturated fatty acid desaturation and elongation pathways in the fish liver (Tocher, Bell, MacGlaughlin, McGhee & Dick 2001; Tocher 2003).

Compared with other plant protein ingredients, commonly used in salmonid diets, white lupin seeds contain a larger amount of linolenic fatty acid (18:3n-3), approximately 9% of the total fatty acids (Yanez *et al*. 1983; Grela & Gunter 1995; Petterson *et al*. 1997). However, around 50% of the total fatty acids of white lupin seed is 18:1n-9 and more than 17% is 18:2n-6 (Yanez *et al*. 1983; Petterson *et al*. 1997). These fatty acids could either cause some physiological metabolic and health problems in the fish or affect the nutritional value of the fish flesh for human consumption, when they are present in commercial fish feed at high concentrations.

The aim of this study was to evaluate the effect of high inclusion levels of whole grain lupin in rainbow trout extruded diets on growth, nutrient utilization and fatty acids in the muscle.

Materials and methods

Diets

Four experimental extruded diets were evaluated: a control diet (L0) and three diets containing 30%, 40% and 50% of whole white lupin grain (*Lupinus albus* var Hamburg) (L30, L40 and L50 respectively). These diets were formulated to replace 0%, 25%, 33% and 42% of the total crude protein of the diet with lupin protein. The diets were formulated using the software SINGLE-MIX® for Windows (FORMAT International, London, UK) and were manufactured by BioMar Chile S.A. (Rancagua, Chile). All diets were formulated to be isonitrogenous and isoenergetic on a digestible basis, but consideration was also given to meet the minimum requirements of essential amino

Table 1 Composition of the ingredients used in this study

Chemical composition (g kg^{-1} DM)	LT fish meal	Whole grain white lupin cv Hamburg	Wheat	Sunflower defatted meal	Feather meal
Dry matter	908	912	878	890	900
Crude protein	680	340	100	370	820
Crude lipid	106	95	20	22	70
Ash	140	36	14	79	29
Carbohydrates*	74	529	866	529	81
Gross energy (MJ kg^{-1} DM)	215.1	208.8	180.5	187.0	235.1
Arginine	39	39	6	32	55
Histidine	25	8	3	9	10
Isoleucine	28	17	4	16	39
Leucine	49	26	8	24	68
Lysine	52	18	3	13	22
Methionine	19	3	2	8	6
Phenylalanine	27	14	6	18	41
Threonine	28	13	3	14	38
Valine	33	15	5	19	59

*Calculated as the remainder of crude protein+crude lipid+ash.

acids, extrudable starch and moisture content. Chromium oxide (Cr_2O_3) was used as an inert marker.

The compositions of all of the ingredients used in the diet preparations are presented in Table 1, and the formulation and proximate composition of the diets are presented in Table 2. The fatty acid and amino acid profiles are shown in Tables 3 and 4 respectively.

Growth experiment

The growth trial was carried out at the experimental fish farm of the Catholic University of Temuco (Los Laureles, Chile). Juvenile rainbow trout (*Oncorhynchus mykiss*) (initial mean weight of 54.0 ± 6.2 g) were randomly distributed into twelve 500 L circular fibreglass tanks (49 fish tank^{-1}) supplied with freshwater (14.0 ± 2.7 °C; flow rate 12 L min^{-1}). Before the start of the growth experiment, the fish were acclimated for 10 days and fed a control diet. Subsequently, triplicate groups of fish were fed the experimental diets by hand, to apparent visual satiety twice a day, for 11 weeks.

At the beginning of the growth experiment, 15 fish were randomly sampled in order to determine the fatty acid profile, body composition and histology. At the end of the experiment, the fish were fasted for a day, and then weighed individually. Two fish from each tank (six per treatment) were randomly taken for whole body composition and stored at −20 °C for proximate analysis. An additional three fish were removed from each tank (nine per treatment) and

Table 2 Ingredient and chemical composition of the experimental diets

	Diets			
	L00	L30	L40	L50
Ingredient composition (g kg^{-1})				
LT fish meal*	400.0	350.0	300.0	250.0
Fish oil*	145.2	136.2	134.0	131.6
Whole grain white lupin†	0	300.0	400.0	500.0
Wheat flour‡	150.0	63.5	63.5	63.4
Sunflower defatted meal‡	138.1	49.0	0.00	0.00
Feather meal§	122.6	85.4	107.5	102.5
Vitamin and mineral premix¶	5.0	5.0	5.0	5.0
Monocalcium phosphate‖	0.3	5.7	10.5	14.3
L-Lysine**	0.2	0.5	1.9	3.1
DL-methionine**	0.0	1.4	2.4	3.1
Chromium oxide††	10.0	10.0	10.0	10.0
Chemical composition (g kg^{-1} DM)				
Dry matter	918.9	927.7	919.0	911.2
Crude protein	491.3	527.7	527.0	484.4
Crude lipid	258.1	254.1	243.7	223.0
Ash	85.3	77.6	72.4	71.0
Carbohydrates‡‡	165.3	140.7	156.9	221.6
Gross energy (kJ kg^{-1})	24.09	24.49	24.30	23.05

*Exapesca S.A. (Talcahuano, Chile).
†Sel Chile S.A. (Temuco, Chile).
‡Graneles Chile S.A. (Santiago, Chile).
§Terramar Chile S.A. (Santiago, Chile).
¶BioMar Chile S.A. (Puerto Montt, Chile).
‖Aquafarma S. A. (Santiago, Chile).
**Evonik Degussa.
††Sigma-Aldrich (St Loius, MO, USA).
‡‡Calculated as the remainder of crude protein+crude lipid+ash.

Table 3 Fatty acid compositions of the experimental diets containing increasing levels of whole grain lupin (as g 100 g^{-1} of the total fatty acids)

	Diets			
	L00	L30	L40	L50
Total lipid (g kg^{-1})	237.2	235.7	224.0	203.2
Fatty acids (g 100 g^{-1} of the total fatty acids)				
C14:0	5.19	5.87	5.82	5.43
C15:0	0.76	0.81	0.78	0.73
C16:0	19.26	19.22	18.64	17.46
C17:0	1.26	1.15	0.90	0.82
C18:0	5.19	2.68	0.49	0.46
C16:1	4.70	5.10	4.81	4.66
C18:1n-9	22.71	25.02	29.27	32.92
C20:1	8.19	8.34	7.91	7.87
C22:1n-9	0.00	1.17	0.92	0.85
C18:2n-6	4.70	5.89	6.08	5.84
C18:3n-3	0.71	0.81	1.30	2.23
C20:4n-6	0.97	0.51	0.10	0.17
C20:5n-3	9.87	9.52	8.68	8.05
C22:6n-3	11.03	10.01	9.25	8.25
Sum SAFA*	34.43	31.29	29.37	26.81
Sum MUFA*	37.08	40.71	43.93	47.37
Sum n-3 PUFA*	21.75	20.46	19.34	18.64
Sum n-6 PUFA*	3.37	2.84	2.74	2.73
n-3/n-6	6.5	7.2	7.1	6.8

*Includes unlisted fatty acids: SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

samples of dorsal muscle were dissected, skinned, deboned, homogenized and stored at -20 °C for fatty acid analysis. These same fish were later sampled for liver weight determination, and both liver and intestine samples were collected for histological examination. Fish were killed by a blow to the head after an overdose of benzocaine (BZ-20, Veterquimica Laboratories, Santiago, Chile).

Digestibility experiment

The digestibility trial was carried out at the school of aquaculture of the Catholic University of Temuco (Temuco, Chile). Apparent digestibility coefficients (ADC) were determined using the modified Guelph method, using Cr_2O_3 as an inert indicator (Cho, Cowey & Watanabe 1985). Thirty Juvenile rainbow trout (*O. mykiss*) (initial mean weight of 181.9 ± 3.4 g) were randomly allocated into twelve 500 L cylindroconical fibreglass tanks equipped with faecal settling columns connected to the outlet of each tank and supplied with well water (14.0 ± 2 °C; flow rate 12 L min^{-1}). Fish were fed the experimental diets in triplicate by hand, to apparent visual satiety twice a day, for 4 weeks.

Table 4 Essential amino acid (EAA) requirement of rainbow trout (*Oncorhynchus mykiss*) compared with the amino acid profile of the experimental diets (expressed as g 100 g^{-1} diet)

		Diets			
Amino acids	Trout EAA requirement*	L00	L30	L40	L50
Methionine	1.40†	0.97	1.04	0.99	0.72
Lysine	1.80	2.77	2.49	2.37	2.20
Threonine	0.80	1.88	1.92	1.93	1.71
Arginine	2.00	2.94	2.77	2.92	3.18
Isoleucine	0.80	1.97	2.01	2.08	1.85
Leucine	1.40	3.38	4.47	4.72	3.63
Valine	1.30	2.51	2.46	2.49	2.15
Histidine	0.70	1.28	1.26	1.24	1.09
Phenylalanine	1.80‡	2.04	2.29	2.35	1.96
Cysteine		0.58	0.65	0.67	0.58
Glycine		3.06	2.76	2.69	2.43
Serine		2.62	2.72	2.79	2.55
Alanine		2.56	2.98	3.01	2.30
Aspartic acid		3.85	3.86	3.94	3.75
Glutamic acid		6.30	7.34	7.66	6.83

*Hardy (2002).
†Methionine+cysteine.
‡Phenylalanine+tyrosine.

After an adaptation period to the dietary treatments of 1 week, faeces were collected daily in each tank from settling columns, centrifuged at 1235 *g* for 15 min and frozen at -20 °C until analysis.

Calculations

Growth was assessed using the thermal growth coefficient (TGC) and weight gain (*G*). Both variables were determined using the following equation: $TGC = [(W_f^{1/3} - W_i^{1/3})]/\Sigma[T \times D] \times 100$, and $G = (W_f - W_i)$, where W_i and W_f are the initial and the 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: $FCR = F \times G^{-1}$, and protein efficiency ratio (PER) was determined as: $PER = G \times P_I^{-1}$, where F is consumption of dry matter from feed, G is the weight gain and P_I is the protein intake. The Hepatosomatic index (HSI) was calculated as: $HSI = 100 \times (LW \times FW^{-1})$, where LW and FW represent wet liver weight and wet body weight respectively.

Apparent digestibility coefficient was determined using the indirect method, as described by Cho *et al.* (1985), dry matter ADC (%) were calculated as $= 100 \times [1 - (\%I_{feed}/\%I_{faeces})$ and ADC of the nutrients as $(\%) = 100 - [100\ (\%I_{feed}/\%I_{faeces}) \times (\%N_{feed}/\%N_{faeces})]$, 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 $DE = (F_{protein} \times ADC_{protein} \times E_{protein}) + (F_{lipid} \times ADC_{lipid} \times E_{lipid}) + (F_{nitrogen\text{-}free\ extract} \times ADC_{nitrogen\text{-}free\ extract} \times E_{nitrogen\text{-}free\ extract})$, 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 ($kJ\,g^{-1}$).

Chemical analyses

Proximate compositions (crude protein, crude lipid, ash and moisture) of diets and carcass were determined according to the methods of AOAC (1998). Carbohydrates were calculated by difference. Gross energy was estimated using the following coefficients: 23.4 $kJ\,g^{-1}$ for crude protein, 39.8 $kJ\,g^{-1}$ for crude lipid and 17.2 $kJ\,g^{-1}$ for carbohydrates (Cho, Slinger & Bayley 1982).

The extraction of total lipids from diets and muscle tissues was carried out according to the method of Folch, Lees and Sloane-Stanley (1957). A sample of 1 g was homogenized in a chloroform/methanol solution (2:1; v/v) and 0.01% butyl hydroxytoluene. Fatty acids methyl esters (FAME) were prepared by acid catalysis transmethylation of total lipids (Morrison & Smith 1964) and analysed by gas chromatography (Hewlett Packard 6890 series II Plus, Wilmington, NC, USA) using a FID detector and a fused silica capillary column (SP-2398, 30 m, 0.25 mm i.d., 0.20 μm film thickness). Helium was used as a carrier gas. Fatty acids were identified by comparison with fatty acid standards (Supelco 37 component FAME mix, Supelco, Bellefonte, PA, USA) and expressed as the percentage of total fatty acids identified.

The total amino acid contents of the experimental diets were determined using near-infrared reflectance spectroscopy by Evonik Degussa (Hanau, Germany). Samples were ground to a 300 μm particle size before analyses.

Histological analysis

Liver and intestine samples were fixed in Bouin solution for 24 h, and stored in 70% ethanol at 4 °C. The tissues were subsequently dehydrated according to standard histological techniques in a graded ethanol series, and embedded in paraffin. Sections (4–6 μm) were cut and stained with haematoxylin and eosin and then blindly examined under a light microscope (Leica Microsystems model DM750, Leica, Bannockburn, IL, USA).

Statistical analyses

Second-order polynomial regression models were used to describe the effects of whole lupin grain dietary inclusion on different parameters studied. The coefficient of determination (R^2) was used to evaluate the total variance explained by the model. The significance level was set to $P < 0.05$. All statistical analyses were conducted using SAS for Windows version 8.01 (SAS Institute, Cary, NC, USA), and data are presented as mean ± standard error of mean.

Results

Growth performance and feed utilization

During the growth trial, all diets were well accepted by fish and the survival rate was 98%. The results of growth performance and feed utilization are presented in Table 5. The mean treatment final body weights varied from 173.6 (L50) to 183.2 g (L40); fish fed the diet containing 500 $g\,kg^{-1}$ of whole lupin grain (L50) achieved the lowest weight gain (119.63 g) and the highest FCR (1.38), although there

Table 5 Growth performance, protein efficiency ratio and Hepatosomatic index of rainbow trout (*Oncorhynchus mykiss*) fed increasing dietary whole grain lupin levels*

Growth performance	Diets				SEM	P_{model}	R^2
	L00	L30	L40	L50			
Initial weight (g)	54.0	54.0	54.0	54.0			
Final weight (g)	178.0	181.9	183.2	173.6	5.53	0.1689	0.33
Gain (g)	124.0	127.9	129.3	119.6	5.52	0.1690	0.33
Feed intake ($g\,fish^{-1}$)	169.3	172.8	180.0	163.2	11.58	0.4604	0.16
TGC	0.13	0.13	0.13	0.13	0.00	0.1545	0.34
FCR	1.28	1.26	1.32	1.38	0.09	0.2512	0.26
PER	1.60	1.64	1.57	1.57	0.13	0.8312	0.04
HIS	1.27	1.45	1.34	1.19	0.06	0.0018	0.75

*Each value is the mean of three replicates.

TGC, thermal growth coefficient; FCR, food conversion ratio; PER, protein efficiency ratio; HSI, Hepatosomatic index.

were no significant correlations with the dietary inclusion of whole lupin grain. Correspondingly, no significant correlations were observed between the increase in the level of whole lupin grain in the feeds and feed intake, TGC and PER ($R^2 = 0.16$, 0.34 and 0.04 respectively).

Whole body composition and HSI

The whole body composition and HSI are shown in Table 6. In general, the inclusion level of lupin in the diet did not change the chemical composition of the fish. Hepatosomatic index, however, showed a significant decreasing trend ($R^2 = 0.75$, $P < 0.05$). The HSI values ranged from 1.19 (L50) to 1.45 (L30).

Histology examination

The cellular morphology of enterocytes showed a displacement of the nucleus in the direction of the distal cell pole. Simultaneously, a reduction in the number of basophils and abundant number of lipid drops were also observed in the fish fed the diet containing 40% whole grain lupin meal (L40) (Fig. 1). Hepatocytes, on the other hand, showed a slight lipid infiltration in the fish fed the control and L40. Surprisingly, the above effect was observed to a lesser degree in the treatment L50 (Fig. 2).

Digestibility coefficients

The ADC are shown in Table 7. The ADC values for dry matter, crude protein, lipid and carbohydrates showed slight variations among dietary treatments, but significant effects of increasing dietary whole lupin grain were not observed.

Protein digestibility ranged from 92.42% (L50) to 94.83% (L40) and lipid digestibility ranged from 98.62% (L50) to 99.06% (L30), whereas carbohydrate digestibility was high, considering the high incorporation of plant ingredient into the diets, and fluctuated from 66.6% (L50) to 75.9% (L40). Phosphorus digestibility was also not affected by the dietary inclusion of whole lupin grain, varying from 62.3% (L30) to 75.2 (L40).

Muscle fatty acid composition

The fatty acid profiles in muscle of rainbow trout before starting the experiment and after feeding the experimental diets are shown in Table 8. The incorporation of high levels of whole grain lupin into diets did not lead to significant changes in the total content of saturated fatty acids (SAFA) ($R^2 = 0.43$, $P = 0.0788$). Among the SAFA, the most abundant fatty acid was the palmitic acid (C16:0), which showed a slight decrease in the diets containing lupin; however, this trend was not significant ($R^2 = 0.38$, $P = 0.1149$).

Total mono-unsaturated fatty acids (MUFA) showed an increase in the muscle as the dietary lupin inclusion was increased. The change in the muscle content of MUFA was due to a significant increase in the oleic fatty acid observed in the fish fed graded lupin diets ($R^2 = 0.69$, $P = 0.0054$).

The total concentration of n-3 PUFA showed a slight decrease as the dietary lupin inclusion was increased; nevertheless, this tendency was not significant ($R^2 = 0.41$, $P = 0.0906$). There was a strong positive quadratic relationship between dietary lupin inclusion and the linolenic acid (C18:3n-3) in the muscle ($R^2 = 0.97$, $P < 0.0001$). On the contrary, a significant negative quadratic relationship was observed ($R^2 = 0.82$, $P = 0.0004$) between the dietary

Table 6 Whole body composition in rainbow trout (*Oncorhynchus mykiss*) fed diets (g kg^{-1} wet weight) with increasing dietary whole grain lupin levels, at the onset and after 120 days of feeding*

		Diets						
	Initial†	L00	L30	L40	L50	SEM	P_{model}	R^2
Dry matter	222.07 ± 0.15	258.10	259.47	260.83	256.10	0.44	0.5576	0.12
Crude protein	185.62 ± 1.14	196.62	195.11	194.34	191.85	0.45	0.4544	0.16
Crude lipid	16.98 ± 0.28	46.92	49.52	50.33	46.14	0.60	0.6812	0.08
Ash	16.16 ± 0.44	13.55	13.65	13.32	13.70	0.04	0.9301	0.02

*Each value is the mean of three replicates (three fish per replicate).
†Initial values are expressed as means ± SEM.

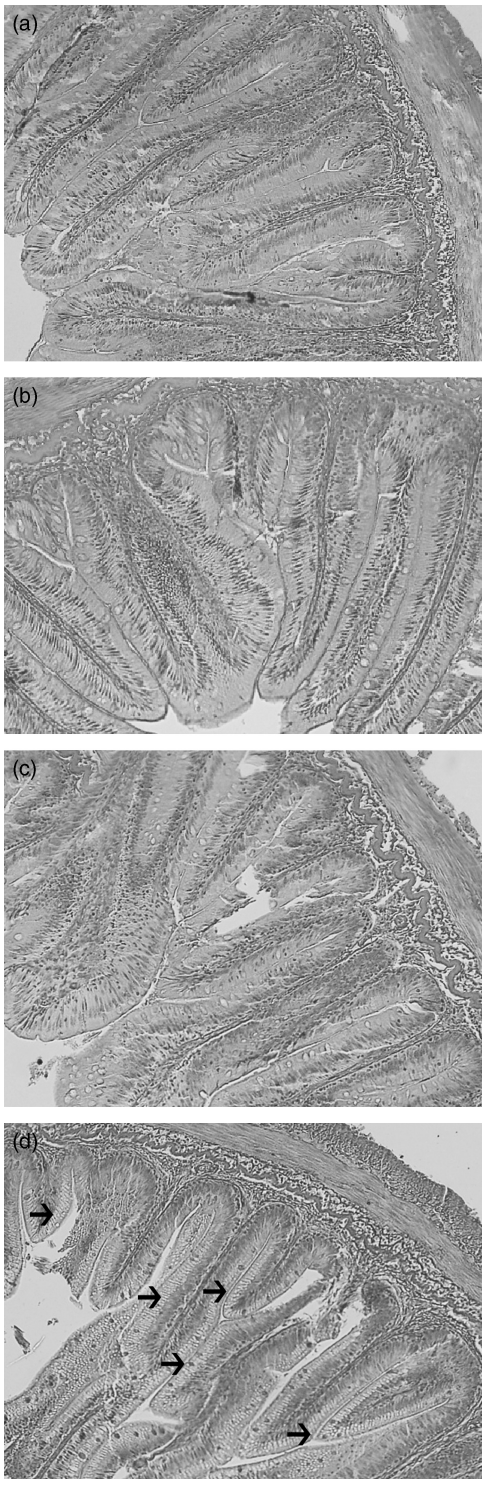

Figure 1 Cross section of middle intestine of rainbow trout fed control diet (a) 30% whole grain lupin meal (b) 40% whole grain lupin meal (c) and 50% whole grain lupin meal (d) (H&E x10). Arrows show an increasing lipid accumulation in the absorptive vacuoles of fish fed the L50 diet.

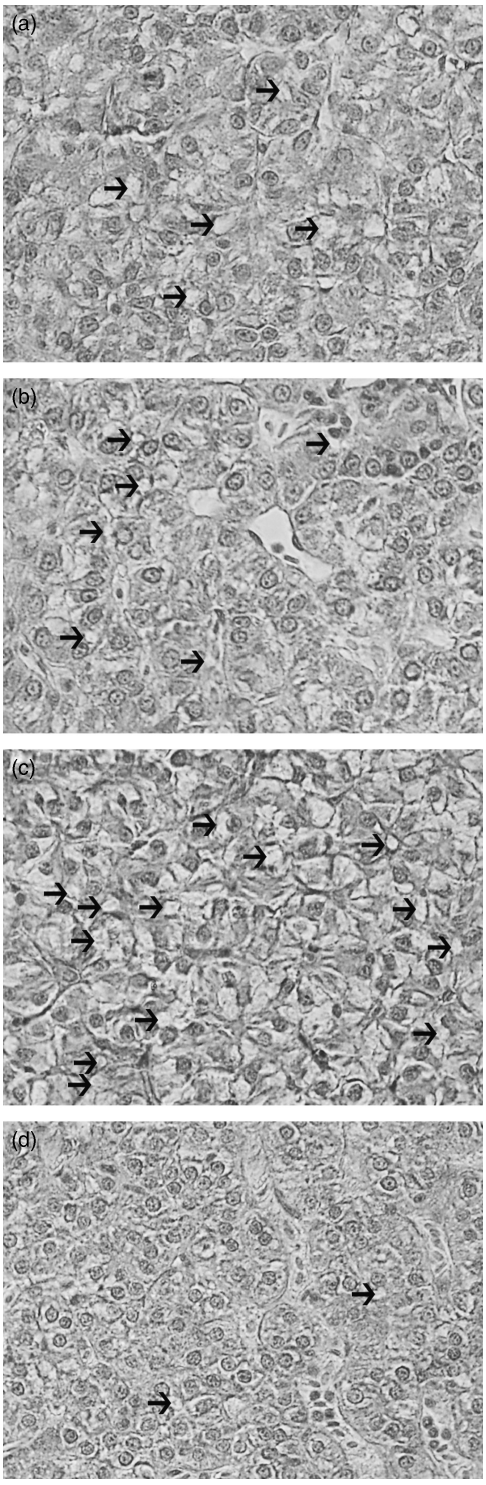

Figure 2 Hepatocytes of rainbow trout fed control diet (a) 30% whole grain lupin meal (b) 40% whole grain lupin meal (c) and 50% whole grain lupin meal (d) (H&E x40). Notable is the higher incidence of lipid vacuolization (indicated by black arrow) in the hepatocytes of fish fed the L40 diet.

Table 7 Nutrient and energy digestibility (%) in rainbow trout (*Oncorhynchus mykiss*) fed increasing dietary whole grain lupin levels*

	Diets						
	L00	L30	L40	L50	SEM	P_{model}	R^2
Dry matter	86.24	86.73	88.97	82.85	2.12	0.0757	0.44
Crude protein	93.09	93.58	94.83	92.42	1.28	0.3391	0.21
Crude lipid	98.51	99.06	98.64	98.62	0.67	0.6729	0.08
Phosphorus	66.31	62.33	75.19	72.69	4.52	0.6561	0.09
Carbohydrates†	69.70	68.39	75.90	66.60	4.77	0.0746	0.44
DE (MJ kg^{-1} diet)	22.42	22.94	22.96	21.10	0.40	0.0007	0.80

*Each value is the mean of three replicates.
†Calculated as the remainder of crude protein+crude lipid+ash.
DE, digestible energy.

Table 8 Fatty acid composition in muscle of rainbow trout (*Oncorhynchus mykiss)* fed diets with increasing dietary whole grain lupin levels, at the onset and after 120 days of feeding (as g 100 g^{-1} of the total fatty acids)*

		Diets						
	Initial†	L00	L30	L40	L50	SEM	P_{model}	R^2
Total lipid (g kg^{-1})	16.8 ± 1.3	42.7	35.2	37.9	33.9	0.76	0.4273	0.17
Fatty acids (g 100 g^{-1} of the total fatty acids)								
C14:0	3.18 ± 0.05	3.76	5.82	5.27	5.23	1.50	0.2984	0.24
C15:0	0.57 ± 0.01	0.72	0.80	0.73	0.75	0.07	0.7241	0.07
C16:0	25.76 ± 1.74	21.96	25.53	22.99	21.99	1.99	0.1149	0.38
C17:0	0.83 ± 0.03	0.74	0.97	0.80	0.79	0.18	0.3338	0.22
C18:0	4.82 ± 0.01	4.43	4.79	4.43	4.02	0.24	0.0145	0.61
C16:1	4.39 ± 0.28	6.02	5.35	5.70	5.46	0.36	0.1565	0.34
C18:1n-9	21.39 ± 0.91	27.00	24.72	28.05	29.65	1.32	0.0054	0.69
C20:1	0.00 ± 0.00	4.98	3.86	4.17	3.84	0.38	0.0114	0.63
C22:1n-9	0.19 ± 0.00	0.76	0.75	0.72	0.57	0.12	0.0798	0.43
C18:2n-6	7.88 ± 0.37	4.81	5.71	5.87	5.98	0.28	0.0009	0.79
C18:3n-3	1.09 ± 0.15	0.67	0.66	0.95	1.56	0.08	<0.0001	0.97
C20:4n-6	0.23 ± 0.02	0.10	0.27	0.09	0.49	0.22	0.2917	0.24
C20:5n-3	4.76 ± 0.18	6.19	5.16	4.79	4.60	0.34	0.0004	0.82
C22:6n-3	20.81 ± 0.51	13.74	11.94	11.13	11.19	1.76	0.1744	0.32
Sum SAFA‡	37.40 ± 1.04	33.10	39.21	35.82	34.16	2.90	0.0788	0.43
Sum MUFA‡	26.66 ± 1.36	40.64	36.35	40.34	41.29	1.84	0.0329	0.53
Sum n-3 PUFA‡	26.83 ± 0.58	20.87	18.02	17.11	17.60	1.98	0.0906	0.41
Sum n-6 PUFA‡	9.10 ± 0.25	5.11	6.13	6.30	6.65	0.44	0.0055	0.69
n-3/n-6	2.95 ± 0.15	4.08	2.95	2.73	2.69	0.43	0.0041	0.71

*Each value is the mean of three replicates (three fish per replicate).
†Initial values are expressed as means ± SEM.
‡Includes unlisted fatty acids: SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

inclusion level of lupin and the muscle concentrations of EPA (C20:5n-3). Surprisingly, the concentration of DHA (C22:6n-3) remained steady in the muscle.

On the other hand, the total concentration of n-6 PUFA increased quadratically with an increase in the dietary inclusion of lupin ($R^2 = 0.69$, $P = 0.0055$). Correspondingly, the content of linoleic acid (C18:2n-6), the main n-6 PUFA, increased in response to the increasing contribution of lupin lipids to dietary crude fat ($R^2 = 0.79$, $P = 0.0009$).

Given the decrease in n-3 PUFA and the increase in n-6 PUFA observed in the muscle of the fish feed graded lupin diets, the n-3/n-6 ratio was reduced from 3.9 in the control diet to 2.42 in the diet containing 50% lupin ($R^2 = 0.71$, $P = 0.0041$).

Discussion

The findings concerning growth performance and nutrient digestibility obtained in the present study have demonstrated the potential use of whole grain sweet white lupin (*L. albus*) in commercial extruded diets for rainbow trout (*O. mykiss*). The growth performances and feed intake of fish fed diets containing up to 50% of whole lupin grain were comparable to those of fish fed the control diet. Similar results were obtained by Burel *et al.* (1998) using dehulled white lupin kernel meal (*L. albus*) and by Glencross, Evans, Hawkins and Jones (2004) using dehulled yellow lupin kernel meal (*Lupinus luteus*) at the inclusion levels of 50%. The results in our experiment are also similar to those achieved by Farhangi and Carter (2001) using dehulled blue lupin kernel meal (*Lupinus angustifolius*), although at slightly lower inclusion levels of 40%.

High inclusions of lupin seed in diets for salmonids in general have been associated with a reduction in the growth rate and feed intake attributable to the deficiency in the essential amino acids (lysine and methionine) (De la Higuera *et al.* 1988) and the presence of antinutritional factors (Francis *et al.* 2001). In this trial, lysine and methionine amino acids were supplemented in the experimental diets in order to cover fish needs (Hardy 2002). Moreover, white lupin cultivar Hamburg, used in the formulation, is characterized by a low alkaloid content (120 mg kg^{-1}), and therefore the effects on palatability reported at higher alkaloid concentrations (Glencross, Evans, Rutherford, Hawkins, McCafferty, Dods, Jones, Harris, Morton, Sweetingharn & Sipsas 2006) were not observed.

The ADC of nutrients and dry matter were not affected by the dietary treatments. The ADC values observed in our study are consistent with many other studies, which included dehulled lupin kernel meal in diets for salmonids (Burel, Boujard, Tulli & Kaushik 2000; Carter & Hauler 2000; Aslaksen *et al.* 2007; Glencross, Hawkins *et al.* 2007, 2008).

The DE of pulses has been considered to be low when fed to fish; this has been attributed to the high content of carbohydrates (Morales, Cardenete, De la Higuera & Sanz 1994). However, the DE values obtained in the present study were relatively high compared with other studies with pulses (Gomes *et al.* 1993); the above is probably due to the high ADC value of carbohydrates achieved by the experimental diets.

Carter and Hauler (2000) reported that in diets for Atlantic salmon (*Salmo salar*), the incorporation of lupin showed the lowest PER value compared with soybean and pea at the same inclusion levels. Conversely, the PER was not affected by the increasing dietary inclusion of whole lupin grain, and the values were higher than those reported by these authors.

The high inclusion of vegetable ingredients in carnivorous fish diets can increase glycogen deposition in the liver after long feeding periods (Russell, Davies, Gouveia & Tekinay 2001). This increase in gluconeogenesis has been related to the low carbohydrate digestibility (Brauge, Medale & Corraze 1994; Hemre *et al.* 2002). Indeed, Farhangi and Carter (2001) suggested an increment in the glucogenic activity as the inclusion levels of lupin increased in the diet. However, in our experiment, the HSI was decreased by dietary treatments, and according to histological examination, no significant decline occurred in glycogen and lipids. These observations are in agreement with those of Robaina *et al.* (1995), who found no alterations in lipid and glycogen storage in hepatocytes from *Sparus aurata* fed with an inclusion of up to 30% of dehulled lupin seed meal.

Histological analysis of the fish fed diets containing 40% and 50% of whole grain lupin exhibited morphological changes in the mid intestine that included a decrease in the number of basophil granulocytes, distal displacement of enterocyte nucleus and an increment in lipid drops. Several authors have reported histological alterations in the intestine such as a shortening of mucosal folds, a loss of the normal supranuclear vacuolization of the absorptive cells in the intestinal epithelium; a widening of the central stroma within the mucosal folding, with increased amounts of connective tissue; and a profound infiltration of inflammatory cells in the lamina propria when plant ingredients are fed to carnivorous fish (Krogdahl, Bakke-Mckellep, Roed & Baeverfjord 2000; Refstie, Korsoen, Storebakken, Baeverfjord, Lein & Roem 2000).

In the case of dehulled lupin meal, Farhangi and Carter (2001) have observed that the increasing dietary inclusions of *L. angustifolius* can slightly shorten the villous height in rainbow trout. Glencross *et al.* (2004) found no effect of the dietary inclusion of yellow lupin (*L. luteus*) on the histology of the intestine in rainbow trout, even though the lupin fed fish had gastrointestinal weights that were higher than those fed other plant ingredient.

The fatty acid composition in rainbow trout muscle was directly related to the fatty acid profile in the respective diets. These results are in agreement with

previous studies using plant ingredients in fish diets (Gomes *et al.* 1993; Caballero, Obach, Rosenlund, Montero, Gisvold & Izquierdo 2002; De Francesco *et al.* 2004; Morris *et al.* 2005; De Francesco, Parisi, Perez-Sanchez, Gomez-Requeni, Medale, Kaushik, Mecatti & Poli 2007; Karalazos, Treasurer, Cutts, Alderson, Galloway, Albrektsen, Arnason, MacDonald, Pike & Bell 2007).

Increasing the dietary inclusion of lupin produced a slight decrease in the total percentage of SAFA, although this was not statistically significant. This can be explained by the reduction in palmitic acid in the diets with higher levels of lupin. Similar results were also found on increasing the inclusion of co-extruded products of rapeseed and peas in the diets fed to rainbow trout (Gomes *et al.* 1993).

Conversely, the MUFA concentrations in muscle are increased on increasing the dietary inclusion of lupin. This is due to an increase in the dietary concentration of 18:1n9, fatty acid found in appreciable amounts in plant ingredients (Yanez *et al.* 1983; Petterson *et al.* 1997; Petterson 1998), causing its accumulation in the muscle, because the desaturation of this fatty acid is limited in the presence of high dietary levels of 18:3n3 and 18:2n6 fatty acids (Tocher *et al.* 2001).

The inclusion of plant proteins (rich in n6 fatty acids) in diets for rainbow trout causes a decreased level of long-chain fatty acids (EPA and DHA) and an increase in unsaturated 18-C fatty acids (oleic acid, linoleic acid and linolenic acid) in the tissues of rainbow trout (Gomes *et al.* 1993; De Francesco *et al.* 2004; Morris *et al.* 2005). The above was also observed in the present study between the control diet and the diets containing whole lupin grain. These changes in the unsaturated 18-C fatty acids concentration are the result of enzyme competition between 18:2n-6 and 18:3n-3 (Tocher 2003). Therefore, a high amount of 18:2n-6 in the experimental diet led to low desaturation activity of n-3 PUFA, increasing the total content of n-6 PUFA and reducing the n-3/n-6 ratio in muscle. Gomes *et al.* (1993) De Francesco *et al.* (2004) and Morris *et al.* (2005) also reported a decrease in the n-3/n-6 ratio in rainbow trout fed diets containing plant protein-based diets, caused by an increase in 18:2n-6 and a decrease in the total content of n-3 PUFA.

Surprisingly, the content of DHA (22:6n-3) was not affected by the dietary treatments, which can be explained by the abundance of n-3 PUFA in the diets supplied by the fish oil and fish meal and in a smaller amount by lupin seed and the capacity of freshwater fish to elongate and desaturate the fatty acids 18:3n- 3 to 22:6n-3 (Tocher *et al.* 2001; Tocher 2003).

To conclude, the dietary inclusion of whole lupin seed up to 50% exerted only marginal effects on fish growth, health, apparent nutrient digestibility and muscle fatty acid compositions. This indicates that white lupin seed may be utilized successfully as a feed ingredient in rainbow trout diets without removing the seed coat, avoiding the cost of a preliminary dehulling process. Future work is required to evaluate whole lupin seed in diets for other salmonids.

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