

Phosphorus and nitrogen utilization efficiency in rainbow trout (*Oncorhynchus mykiss*) fed diets with lupin (*Lupinus albus*) or soybean (*Glycine max*) meals as partial replacements to fish meal

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ABSTRACT: The study was conducted to compare two plant ingredients as dietary protein sources for rainbow trout on the basis of feed acceptability, survival, growth, feed conversion, nitrogen and phosphorus utilization efficiency and loading. Two extruded diets were formulated with the inclusion of a soybean meal (DS) and a lupin meal (DL). The control diet was a fish meal (FM) based diet. All diets were isonitrogenous and isolipidic. Triplicate groups of 65 fish (5.10 ± 0.10 g) were assigned to each diet. At the end of the experiment (66 days), all groups of fish fed diets had a similar final growth and feed utilization efficiency ($P > 0.05$). Nitrogen retention rate was higher for the group fed the control diet and in consequence the calculated loading amount of this nutrient resulted lower when compared with the test diets. However, phosphorus retention was higher in the groups of fish fed the experimental diets (DL 26.58 ± 0.22 and DS 27.67 ± 3.05) when compared to the control diet (22.08 ± 1.12) ($P < 0.05$). This represents a phosphorus loading of 8.33 ± 0.23 , 8.96 ± 1.02 , and 11.55 ± 0.67 kg/t production for the diets DL, DS, and control, respectively. Therefore, the results indicate that lupin meal can be used as a possible plant protein source for the formulation of low-phosphorus loading diets for rainbow trout without affecting feed acceptability and growth performance. This legume represents a clear opportunity to supply the high demand for plant protein sources for aquaculture. Further studies are needed to evaluate and compare different lupin species and varieties.

Keywords: sustainable aquaculture; alternative ingredients; phosphorus loading; growth performance; phosphorus retention

INTRODUCTION

Aquaculture is considered the fastest growing food-producing sector in the world and is set to play a key role in meeting the rising demand for fishery products (FAO 2014). While the importance of this industry is accepted and encouraged, it also requires the generation of new strategies that ensure its sustainability and reduction of any negative impact over the environment (Hasan 2001). Since the most intensive aquaculture production systems have a high reliance on formulated diets,

the research on nutrition and feeding management strategies must play a key role in the sustainable development of the aquaculture sector (FAO/NACA 2012). Continuing expansion of aquaculture stresses the need for specific feeds formulations and alternative protein ingredients to fishmeal (Tacon and Metian 2008). Furthermore, the goal of efficient and productive feeding of animals, within economic and environmental constraints, is to increase the proportion of ingested nutrients recovered as useful products. For this reason, the simplest way to reduce the excess of nutrients from

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intensive fish culture effluents is modifying the diets to increase retention efficiency of dietary nutrients by replacing conventional raw materials, as fishmeal or soybean meal, with others that offer more highly bioaccessible and bioavailable nutrients. Thus, the impacts of altering the type of nutrient source in some fish species have been widely studied and the implications of using terrestrial plant ingredients and dietary additives to reduce dissolved nitrogen and phosphorus wastes have been recognized (Hernandez et al. 2005, 2012, 2013a, b; Khajepour and Hosseini 2012; Sarker et al. 2012). Proportional replacement of fish meal by plant-based ingredients is impeded by the poor growth response frequently seen in fish fed high levels of plant raw materials (Geurden et al. 2013). Still, some plant protein ingredients have been described as feasible alternatives for partial replacement of fishmeal in diets for aquaculture. Our previous experiments indicate that an adequate combination of alternative plant low-phosphorus protein ingredients, mainly soybean meal, significantly reduces the phosphorus loading from rainbow trout diets without compromising the growth (Sato et al. 2003; Hernandez et al. 2004).

Among other potential plant protein ingredients, lupin has been described as a possible alternative for partial replacement of fishmeal in diets for aquaculture in countries like Australia, Chile, and the European Union. Interest in lupin as alternative protein source has increased, and research and development efforts aiming to improve the available varieties have intensified. This legume offers economic and functional advantages over the use of other plant protein sources, such as soybean (Glencross et al. 2011). Furthermore, biologically active compounds present in lupin seeds additionally increase the nutritional value of this plant not only as a high protein material but also as a rich source of natural antioxidants for disease prevention and health promotion (Lampart-Szczapa et al. 2003; Sbihi et al. 2013; Czubinski et al. 2014). Many studies have reported that meals from different lupin species can be used to substitute fishmeal in aquafeeds without significant deleterious effects on growth performance, nutrient digestibility, health condition, and muscle composition of fish (Borquez et al. 2011a, b; Glencross et al. 2011; Tabrett et al. 2012; Hernandez et al. 2013a; Glencross et al. 2014; Salini and Adams 2014). Despite that, soybean meals are used more extensively in aquafeeds, and their

effects on fish digestive function and intestinal integrity have been investigated in detail. It has been shown that soy meals induce inflammation and pathomorphological changes in the distal intestine of salmonid fish together with reduction of digestive functionality such as lesser activity of enzymes (Refstie et al. 2000, 2010). Some studies have indicated that different lupin kernel meals or protein concentrates do not alter the morphology of the fish intestine making this ingredient more suitable for the formulation of aquafeeds than soybean (Refstie et al. 2006; Omnes et al. 2015).

In this study we aim to gather preliminary information for the development of low-phosphorus loading diets for rainbow trout by comparing the effect of using lupin meal against soybean meal, as alternative plant protein sources to fish meal, over feed acceptability, survival, growth, feed conversion, nitrogen and phosphorus utilization efficiency and loading.

MATERIAL AND METHODS

Experimental diets. The formulation and chemical composition of the experimental diets are shown in Table 1. Soybean and lupin meals were included at the percentages required to supply the same amount of protein. All of the experimental diets were formulated to be isonitrogenous and isoenergetic and contained approximately 46% crude protein and 21 MJ/kg gross energy. The diets were manufactured at the Animal Feed Pilot Plant of the Aquaculture School, Catholic University of Temuco, Chile. Prior to mixing, all solid dry ingredients were finely ground through a 200 µm mesh using an Ultra Centrifugal Mill ZM 200 (Restch GmbH & Co., Haan, Germany). Ingredients were thoroughly mixed prior to the extrusion process. Feeds were prepared by cooking-extrusion in a twin screw extruder Clextral BC-21 (Clextral, Firminy, France) with a 2-mm diameter die. The resultant moist pellets were oven dried at 60°C for approximately 8 h and then coated with fish oil, according to the formulation for each diet, by means of a laboratory vacuum coater VC10 (Dinnissen, Sevenum, the Netherlands). Diets were stored in a freezer at –20°C until use. A representative sample of each diet was analyzed in duplicate to assess the proximate composition.

Fish, feeding, and experimental conditions. Rainbow trout, *Oncorhynchus mykiss* were obtained

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from La Esperanza fish farm (Cunco, Araucania region, Chile) and transferred to cylinder-conical stocking tanks (500 l) at the Aquaculture School, Catholic University of Temuco, Chile. Prior to the start of the feeding trial, the fish were acclimated for five days and fed the control diet. Fish with an average body weight of 5.60 ± 0.10 g were randomly selected from stock and distributed into 100 l fibreglass tanks at a density of 65 fish per tank. The experimental set-up was arranged in a completely randomized design with three replications per treatment and the feeding was conducted for 66 days. Fish were hand fed twice a day to apparent satiation level. The tanks were well aerated and had continuous well water supply at a rate of 1.0–1.6 l/min and an average temperature of $14 \pm 0.4^\circ\text{C}$.

Table 1. Ingredients and proximate composition of the experimental diets for *Oncorhynchus mykiss*

Ingredients (%)	Experimental diets		
	Control	DL	DS
Fishmeal ^a	63	15	15
Defatted soybean meal ^b	0	0	16
Lupin meal ^c	0	25	0
Corn gluten meal ^b	0	15	15
Hydrolyzed feather meal ^b	0	8.5	8.5
Poultry by-products meal ^b	0	8.5	8.5
Wheat meal ^d	10	10	10
Tapioca starch ^e	5	5	5
Fish oil ^b	10	10	12.5
Vitamin-mineral premix	1.5	1.5	1.5
Cellulose ^f	10.5	0.5	7.0
Ca(H ₂ PO ₄) ₂ ^f	0	1	1
Total	100	100	100
Proximate composition (%)			
Dry matter	96.76	95.73	95.60
Total protein	45.91	45.74	47.05
Total lipids	13.08	14.99	12.86
Ash	13.26	8.17	8.72
NFE	19.12	27.13	23.87
Phosphorus	1.73	1.22	1.27
Energy (MJ/kg DM)	20.96	22.32	21.82

DL = lupin meal, DS = soybean meal, NFE = nitrogen free extract, DM = dry matter

^aPesquera San Jose, Talcahuano, Chile; ^bBiomar, Puerto Montt, Chile; ^cSemillas Baer, Temuco, Chile; ^dMolinos Gorbea, Gorbea, Chile; ^eAlmisa, Caarguazu, Paraguay; ^fSigma, St. Louis, USA

Sample collection and chemical analyses. In order to determine growth changes and to calculate feed utilization parameters, fish from each tank were individually weighed at the start and at the end of the feeding trial. Fish were starved for 24 h and anesthetized with isoeugenol (Aqui-S[®]; Bayer Animal Health, Santiago, Chile) prior to weight measurement. Fifteen individuals were randomly sampled from the initial stock of fish and five individuals from each tank at the end of the experimental period. Sampled fish were totally minced using an ultra-centrifugal mill ZM200 (Retsch GmbH & Co.) fitted with 0.5 mm screen and the homogenate was preserved at -20°C for the chemical analyses of whole body.

Growth was assessed using the specific growth rate (SGR), thermal growth coefficient (TGC), and weight gain (G). These variables were determined using the following equations:

$$\text{SGR} = (\ln W_f - \ln W_i) \times 100 / \text{rearing period (days)}$$

$$\text{TGC} = [(W_f^{1/3} - W_i^{1/3}) / \Sigma(T \times D)] \times 100$$

$$G = W_f - W_i$$

where:

W_i = initial weight

W_f = final weight

D = number of feeding days

T = average water temperature

Feed efficiency ratio (FER) and protein efficiency ratio (PER) were calculated as:

$$\text{FER} = G/F$$

$$\text{PER} = G/PI$$

where:

F = consumption of dry matter from feed

G = weight gain

PI = protein intake

Condition factor (CF) was determined as:

$$\text{CF} = (W_f/L_f) \times 100$$

where:

W_f = final weight

L_f = final body length

The proximate composition of ingredients, diets, and carcass samples was determined according to AOAC (1995). Dry matter was calculated by gravimetric analysis following oven-drying at 105°C for 24 h. Protein levels were calculated from

the determination of total nitrogen by Kjeldahl digestion (based on $N \times 6.25$). 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 samples in a muffle furnace at 550°C for 3 h. Fibre content was calculated by gravimetric analysis following oven-drying at 105°C for 24 h and acid and alkali digestion with sulphuric acid and sodium hydroxide, respectively. Nitrogen free extract (NFE) content was determined by difference. The gross energy in the diets was determined using a bomb calorimeter C2000 basic (IKA[®]-Werke GmbH & Co. KG, Staufen, Germany). Phosphorus concentrations were determined using a molybdovanadate reagent and spectrophotometric measurement at 400 nm (Lambda 25 UV/VIS Spectrometer; PerkinElmer, Waltham, USA).

The values obtained from initial and final whole body were employed for calculating P and N retention. The total P and N loading (considering fecal and non-fecal excretion and expressed as kg/t fish production) were based on P and N retention and calculated as the difference between the amount of nutrient consumed and the amount of nutrient retained (Watanabe et al. 1987).

Statistical analyses. For statistical comparisons the effects of dietary treatments on measured parameters were assessed by one-way analysis of variance (ANOVA) using the Minitab software (Version 12.1, 1998) for MS Windows. Differences between means were determined by Tukey's test. Significance was declared tested at $P < 0.05$.

RESULTS

Data on overall growth performance and feed utilization of fish feeding on the experimental diets are summarized in Table 2. Mortality was not significant during the trials and fish remained apparently healthy for all treatments. Trout fed the diets with lupin or soybean meals gained $381.90 \pm 40.80\%$ and $393.60 \pm 14.40\%$ of their initial weights, respectively, which was statistically equal ($P > 0.05$) to the group of fish fed the fishmeal based diet used as control ($376.10 \pm 22.70\%$). Conversely, even if the best results in terms of feed utilization were found in the control group, there were not significant differences with the groups of fish fed diets with lupin or soy. Thus, all tested diets presented similar results and the growth and feed efficiency obtained are acceptable for the size of fish evaluated.

The analysis of carcass showed no significant difference in body composition of juvenile rainbow trout among all the experimental groups ($P > 0.05$) after 66 days of the feeding trial. The whole body crude protein from the initial stock of fish was $12.88 \pm 0.0\%$ and 15.10 ± 0.30 , 13.60 ± 1.0 , and $14.30 \pm 0.50\%$ for fish fed control, DL, and DS diets, respectively. In the case of whole body phosphorus content the initial group of fish presented $0.47 \pm 0.0\%$ and 0.34 ± 0.20 , 0.33 ± 0.10 , $0.35 \pm 0.10\%$ for the groups of fish fed control, DL, and DS diets, correspondingly. The results of phosphorus and nitrogen utilization and estimated loading amounts of the various experi-

Table 2. Growth and feed utilization of *Oncorhynchus mykiss* fed experimental diets

	Control	DL	DS
Initial weight (g)	5.70 ± 0.10	5.50 ± 0.10	5.60 ± 0.10
Final weight (g)	27.10 ± 1.00	26.50 ± 1.90	27.70 ± 1.10
Growth (%)	376.10 ± 22.70	381.90 ± 40.80	393.60 ± 14.40
Feed intake (g/fish)	18.34 ± 0.60	20.66 ± 0.20	21.58 ± 0.70
Feed efficiency ratio	0.86 ± 0.00	0.99 ± 0.10	0.97 ± 0.10
Specific growth rate (%/day)	2.36 ± 0.10	2.38 ± 0.10	2.42 ± 0.00
Thermal unit growth coefficient	1.37 ± 0.00	1.37 ± 0.10	1.41 ± 0.00
Condition factor (%)	1.21 ± 0.20	1.09 ± 0.10	1.03 ± 0.10
Protein efficiency ratio	2.17 ± 0.10	1.90 ± 0.20	1.94 ± 0.10
Final survival rate (%)	99.49 ± 0.90	100.0 ± 0.00	98.97 ± 1.80

DL = lupin meal, DS = soybean meal

each value is the mean \pm standard error of three replicates

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Table 3. Phosphorus and nitrogen retention and loading from rainbow trout fed the experimental diets

Diet	Retention (%)		P loading (kg/t production)	N loading (kg/t production)
	P	N		
Control	22.08 ± 1.12 ^a	39.96 ± 2.56 ^a	11.55 ± 0.67 ^a	37.80 ± 2.29 ^a
DL	26.58 ± 0.22 ^b	33.82 ± 2.31 ^b	8.33 ± 0.23 ^b	45.03 ± 0.19 ^b
DS	27.67 ± 3.05 ^b	31.89 ± 1.44 ^b	8.96 ± 1.02 ^b	49.95 ± 4.46 ^b

DL = lupin meal, DS = soybean meal

Retention (%) = [(final nutrient content in carcass – initial nutrient content in carcass)/dietary nutrient intake] × 100

Nutrient load (kg⁻¹) = [(specific growth rate × nutrient in diet (g) – nutrient retained in fish (g))/production (t)] × 100

each value is the mean ± standard error of three replicates

^{a,b}values with different superscript letters denote significant differences between treatment groups ($P < 0.05$)

mental groups fed the control, DL, and DS diets are shown in Table 3. Dietary phosphorus was efficiently retained by fish fed the diets with lupin (26.58 ± 0.22%) and soybeans (27.67 ± 3.05%), resulting in significantly ($P < 0.05$) lower phosphorus loading values (8.33 ± 0.23 and 8.96 ± 1.02 kg/t of production, respectively) in comparison with the fish meal based diet used as control (11.55 ± 0.67 kg/t of production). Although there were no statistical differences between both diets with the inclusion of plant alternative ingredients, the diet with lupin presented slightly better results in terms of phosphorus retention and loading. The calculation of nitrogen retention gave values that varied between the treatments from 31.89 ± 1.44 to 39.96 ± 2.56%. Higher estimated amounts of nitrogen loaded into the water were observed for the experimental diets that included lupin and soybean meals in comparison with the fishmeal based diet ($P < 0.05$). Among the experimental diets with alternative plant protein ingredient the diet with lupin showed the best results in terms of nitrogen retention and loading (33.82 ± 2.31% and 45.03 ± 0.19 kg/t of production, respectively).

DISCUSSION

The use of alternative plant protein ingredients in the diets tested in the present study resulted in good survival, feed acceptability, growth and feed conversion besides lowering phosphorus loading. Rainbow trout fingerlings fed a low fishmeal based diet formulated with lupin meal (*Lupinus albus*) as alternative plant protein ingredient improved phosphorus retention efficiency without affecting growth and feed acceptance and efficiency. Thus, the experimental results obtained in this feeding

trial show that lupin meal (*Lupinus albus*) has great potential as an alternative protein ingredient from the viewpoint of efficient utilization of dietary protein and phosphorus. These results were in agreement with earlier studies on environmentally friendly feeds for aquaculture emphasizing the importance of retention efficiency of nutrients such as phosphorus and nitrogen for the evaluation of feed quality (Jahan et al. 2003; Satoh et al. 2003; Hernandez et al. 2005).

There are several key facets to determining or placing a nutritional or biological value on a feed ingredient, the principle of which is defining the proportion of nutrients that an animal can obtain from a particular ingredient through its digestive and absorptive processes (Glencross et al. 2007). In this way, not only an improved understanding of the level of variability in the nutrient composition of the ingredient but the knowledge about how different factors can either inhibit or enhance nutrient absorption and/or utilization in the cultured organism are key steps to maximizing the potential value of the ingredient. The identification and evaluation of new alternative ingredients with high protein and phosphorus bioaccessibility and bioavailability is necessary in order to formulate less polluting aquafeeds. The potential bioavailability will be closely dependent on the bioaccessibility, at least for the macronutrients (including protein and phosphorus). Bioaccessibility is directly affected by numerous factors, some of which are more linked to the substrate or ingredient itself, such as the chemical form of the nutrients supplied and/or presence of anti-nutritional factors (Lall 1991). Other host factors as species, gender, age, physiologic state, and coexisting pathologic conditions can also affect bioavailability (Satoh

et al. 2002). Furthermore, physical and thermal processing of the feed ingredients might induce changes in the bioavailability and bioaccessibility of nutrients by changing their supramolecular organization, the network of interactions between molecules or their localization within compartments that are normally inaccessible to digestive enzymes (Bordoni et al. 2014). These changes or modifications will depend on the kind of selected processing method and the complexity of the ingredient matrix. In contrast with the soybean meal, the lupin meal used in the formulation of the experimental diets was not defatted or thermally processed, leaving a margin for future studies aiming to evaluate different methods of processing over the enhancement of the nutritional value of this ingredient for aquafeeds. Proper processing of the lupin meal could probably increase even more the bioavailability and bioaccessibility of protein and phosphorus improving their retention and as a result limiting the amounts discharged to the aquatic environment.

Disproportionate amounts of limiting nutrients could alter the ecological balance of the aquatic system. An excessive concentration of phosphorus is considered the most common cause of eutrophication in freshwater systems. Conversely, in the marine systems, nitrogen is considered to be the key mineral nutrient controlling primary production (Black 2001). To reduce phosphorus excretion in animal production, formulation of diets based on available phosphorus rather than total phosphorus will be necessary (Lynch and Caffrey 1997). Dietary available phosphorus requirement for optimum growth, feed utilization, and bone mineralization ranging from 0.4 to 0.8% has been reported for rainbow trout and other fishes (Sugiura et al. 2000; NRC 2011). The experimental diets evaluated in this study were formulated to contain the required amount of available phosphorus, and at the same time the minimum amount of total phosphorus, which ranged from 1.22 to 1.73%. An important proportion of phosphorus in plant ingredients is present as phytate-phosphorus, with the remaining portion present as inorganic salts or other compounds that are bioavailable to fish. Phytate-rich plant ingredients restrict the bioavailability of phosphorus along with other minerals. The total phosphorus, phytate-phosphorus content, and the proportion of phytate-phosphorus in total phosphorus are usually higher for soybean meals when compared with the

lupins species most commonly used as ingredients for aquafeeds (Kumar et al. 2012).

The results in this study indicate that sweet or debittered varieties of lupin meals, as the one used in this study, can be considered a possible plant protein source for the formulation of low-phosphorus diets for rainbow trout without affecting feed acceptability and growth performance. This legume represents a clear opportunity to meet the high demand for plant protein sources for aquaculture. Further studies are needed to characterize and evaluate nutritional quality, nutrient availability, and ways to improve the use of other sweet lupin species and varieties as a protein source to formulate less polluting aquafeeds by contrasting *in vivo* and *in vitro* methodologies.

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