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Mixtures of lupin and pea protein concentrates can efficiently replace high-quality fish meal in extruded diets for juvenile black sea bream (*Acanthopagrus schlegeli*)

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ABSTRACT

A 60-day feeding experiment was carried out to investigate the effects of including lupin protein concentrate (LPC) and pea protein concentrate (PPC) in multiple essential amino acid-supplemented extruded diets for black sea bream (*Acanthopagrus schlegeli*). Nine diets, including eight diets formulated to contain four mixtures of LPC and PPC (L/P ratio, 3:0, 2:1, 1:2 and 0:3) with two dietary inclusion levels (300 or 500 g plant protein kg^{-1} dietary protein) and one diet with high-quality fish meal as the sole protein source (FM diet) were fed to 18 tanks of 13-g black sea bream. Growth performance, nutrient utilization, and brush-border membrane bound maltase activities were evaluated. An average weight gain (WG) of 32.7 g $fish^{-1}$ and an average feed conversion ratio (FCR) of 1.13 g ingested dry matter (g gain)$^{-1}$ were obtained. Neither plant protein inclusion level nor L/P ratio significantly affected body composition (except ash), fish somatic indices or plasma parameters. The high inclusion of 500 g kg^{-1} resulted in significantly higher FCR than what was obtained with 300 g kg^{-1} inclusion. The WG, whole body ash content, and nitrogen (N) and energy retentions of these fish were, however, significantly lower than that of the fish fed diets with low plant protein inclusion (300 g kg^{-1}). The highest LPC inclusion (L/P ratio = 3:0) resulted in significantly higher feed intake and FCR, and lower N retention than the treatments with less LPC, but did not affect the growth rates or energy retentions. The diet with the highest PPC inclusion resulted in significantly reduced maltase activity in distal intestine. Any combination of LPC and PPC in essential amino acid-supplemented extruded diets, accounting for up to half of dietary protein, can be used without impairing fish growth. At high inclusion, combinations with more PPC are preferred, due to less efficient feed conversion caused by the LPC.

1. Introduction

Aquaculture of black sea bream (*Acanthopagrus schlegeli*) is increasing in East Asia. The main traditional source of feed for this species is 'trash fish', leading to a series of problems such as unbalanced and incomplete nutrient composition, contamination by unsafe transport and handling, water pollution, and growth of pathogenic bacteria. Published nutritional studies on black sea bream have up to now mainly focused on the basic nutrient requirements, including protein, essential amino acids, vitamins and phosphorous (Shao et al., 2008; Peng et al., 2009). There is, however, limited published information concerning the nutritional value of different feed protein ingredients in the diet for black sea bream.

Lupin and pea are legumes with high potentials as sources of protein in diets for salmonid fish (Burel et al., 1998; Carter and Hauler, 2000; Glencross et al., 2002, 2003, 2004a, 2004b, 2008; Refstie et al., 2006). These plant protein sources have also demonstrated their potentials in diets for temperate and warm water fish such as European sea bass (*Dicentrarchus labrax*) (Gouveia and Davies, 1998, 2000; Adamidou et al., 2009), milkfish (*Chanos chanos Forsskal*) (Borlongan et al., 2003), and silver perch (*Bidyanus bidyanus* (Mitchell)) (Booth et al., 2001). In gilthead sea bream, dietary inclusion of unprocessed lupin and pea meals is limited to 20% and 30% of the dietary protein, respectively (Pereira and Oliva-Teles, 2002, 2004). The low dietary inclusion level can be related to the low protein content, imbalanced amino acid composition and presence of anti-nutritional factors (ANF) in the lupin and pea.

Removing the indigestible carbohydrates by extraction from lupin and the starch by air classification from peas, results in lupin (LPC) and pea protein concentrates (PPC) with high nutrient digestibilities, that can be efficiently used in diets for salmonids (Carter and Hauler, 2000; Thiessen et al., 2003; Glencross et al., 2006; Øverland et al., 2009). PPC can provide 40% protein in the diet for gilthead sea bream diet without

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any negative effects on growth and feed utilization when replacing fish meal as a source of protein (Sánchez-Lozano et al., 2011). Combining PPC and rice protein concentrate rather than using PPC alone, allowed an increase of PPC up to 60% of the protein in diets for gilthead sea bream (Sánchez-Lozano et al., 2009). Zhang et al. (2012) showed that any combination of LPC and PPC with multiple essential amino acid-supplementation can be efficiently used when total plant protein inclusion is limited to 300 g kg^{-1} crude protein in extruded diets for rainbow trout (*Oncorhynchus mykiss*). At higher inclusion (500 g kg^{-1}) PPC appeared to be a preferable source of protein.

The aims of the present experiment were to 1) determine the effect of including LPC and PPC in extruded high energy diets on the growth, nutrient utilization and intestinal enzyme activities of juvenile black sea bream, 2) investigate if the combination of these ingredients allowed a higher dietary inclusion rate than when applied separately, and 3) determine the optimal combination of LPC and PPC in extruded diets for juvenile black sea bream.

2. Materials and methods

2.1. Ingredients and diets

The LPC was derived from white lupine (*Lupinus albus*), produced by dehulling, milling, aqueous extraction of lupine seeds to remove sugars and soluble non-starch polysaccharides (NSP), heating and spray-drying. The PPC was produced from yellow field pea (*Pisum sativum* L.) by dehulling, fine grinding and air-classification. The chemical composition of these two plant concentrates and LT fish meal has been previously reported by Zhang et al. (2012). The LPC and PPC were each supplemented with the first-three limiting essential amino acids to balance the essential amino acid profile to that of LT fish meal. A 2×4 factorial design was used in the present experiment, where the factors were inclusion level of plant protein concentrate (300 or 500 g protein kg^{-1} dietary protein), and ratio between essential amino acid-supplemented LPC and PPC in the diets (L/P ratio at 3:0, 2:1, 1:2 and 0:3). The diets were isonitrogenous (530 g crude protein (CP) kg^{-1}) and isolipidic (160 g crude lipid (CL) kg^{-1}). In addition, a diet with LT fish meal as the sole source of protein (FM diet) was produced with 570 g CP and 180 g CL kg^{-1}, and formulated to keep the same ratio between protein and lipid ratio as the 8 diets with plant protein sources. Yttrium oxide was used as a marker for digestibility measurement (Austreng et al., 2000). Feed formulation and chemical composition are shown in Table 1. Feed processing and equipment are described in detail by Zhang et al. (2012). All dry ingredients were ground, mixed, preconditioned and extruded in a twin screw extruder with 2.0 mm dies and the pellets were dried and coated with fish oil in a vacuum coater. Yttrium oxide was added to the diets for determination of nutrient digestibilities, but fecal collection by stripping was not successful, and digestibility results are not presented.

2.2. Fish and feeding

The experiment was conducted at the Joint Laboratory of Nutrition and Feed for Marine Fish, Marine Fisheries Research Institute of Zhejiang Province (Putuo, Zhoushan, China). The black sea bream juveniles were obtained from a hatchery in Fodu (Putuo), acclimated in an indoor concrete pond for three weeks, and fed a commercial diet (52% CP, 8% fat). Before the start of the experiment, 900 bream with an initial weigh of 13 g were depleted of feed for 24 h, anaesthetized with MS-222 (90 mg l^{-1}), batch-weighed, then randomly assigned to 18 circular 500-l tanks, fifty fish per tank. Each tank was supplied with sand-filtered seawater at a flow rate of 1.5 l min^{-1} and additional aeration via air stone. A natural photoperiod (13 h light, 11 h

Table 1
Diet formulation and analyzed chemical composition (based on dry matter).

Diets	FM	LLP1	LLP2	LLP3	LLP4	HLP1	HLP2	HLP3	HLP4
Ingredients, g kg^{-1}									
Fish meal[a]	706.0	436.0	436.0	436.0	436.0	309.0	309.0	309.0	309.0
LPC[b]	–	280.0	186.0	94.0	–	467.0	311.0	155.0	–
PPC[c]	–	–	86.0	173.0	260.0	–	145.0	289.0	433.0
Fish oil[d]	121.0	98.0	103.0	107.0	112.0	94.0	102.0	110.0	118.0
Wheat	160.9	165.0	168.0	170.0	173.0	103.0	108.0	112.0	116.0
Premix[e]	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
Y_2O_3[f]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
L-Lys[g]	–	6.1	5.0	3.9	2.7	10.2	8.3	6.4	4.6
DL-Met[h]	–	3.4	3.3	3.2	3.1	5.7	5.6	5.4	5.2
L-Trp[i]	–	0.6	0.4	0.2	–	1.0	0.7	0.3	–
L-Thr[j]	–	–	0.5	1.0	1.6	–	0.9	1.7	2.6
Analyzed content, kg^{-1}									
Dry matter, g	939	943	934	936	945	936	950	933	940
In dry matter									
Crude protein, g	575	529	534	534	533	522	522	531	528
Crude fat, g	174	163	164	152	143	161	157	152	149
Starch, g	110	100	107	110	121	69	84	98	107
Ash, g	107	78	81	83	89	70	76	78	77
Gross energy, MJ	23.5	23.1	23.1	23.0	22.2	23.0	22.5	22.8	22.1
Phosphorous, g	16.3	11.0	11.3	11.6	12.7	8.9	9.8	10.6	11.2
Phytic acid, IP6, g	1.47	3.02	3.49	4.83	5.61	4.19	4.47	6.07	8.40

[a] Norse LT-94®, low-temperature dried fish meal, Norsildmel, Bergen, Norway.
[b] NaProLup PO54®, Lupin protein concentrate, derived from white lupins (*Lupinus albus*), NaProFood, Bruckberg, Germany.
[c] PPC 55 PELLET, Pea protein concentrate, derived from yellow field pea (*Pisum sativum* L.), AgriMarin AS, Stavanger, Norway.
[d] Silfas, Karmsund, Norway.
[e] Per kg diet: vitamin A: 2000 IU, vitamin D_3: 1200 IU; vitamin E: 160 mg; vitamin K_3: 8 mg; vitamin B_1: 12 mg; vitamin B_2: 20 mg; vitamin B_3: 60 mg; vitamin B_5: 24 mg; vitamin B_6: 12 mg; vitamin B_9: 4 mg; vitamin B_{12}: 0.016 mg; vitamin C: 100 mg; Biotin: 0.2 mg; Ca: 876 mg; Cu: 4 mg; Co: 0.8 mg; I: 2.4 mg; Mn:12 mg; Zn: 96 mg.
[f] Metal Rare Earth Limited, Shenzhen, China.
[g] *L*-Lysine-HCl, 99% feed-grade, CJ Indonesia, Jakarta, Indonesia.
[h] Rhodimet® NP 99, *DL*-methionine, 99% feed-grade, Adisseo Brasil Nutricao Animal Ltda, Sao Paulo, Brazil.
[i] TrypAMINO®, *L*-tryptophan, 98% feed-grade, Evonik Fermas S.R.O., Slovenska Lupca, Slovakia.
[j] *L*-Threonine, 98.5% feed-grade, Ajinomoto Eurolysine S.A.S., Paris, France.