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Utilisation of diets supplemented with microbial phytase by seabass (*Dicentrarchus labrax*) juveniles

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Abstract – A trial was carried out to compare the growth performance, body composition and apparent digestibility coefficients (ADC) of diets by seabass, with an initial weight of 13.5 g, fed diets including fish meal (68.6 % of the dietary protein) or soybean meal (65.6 % of the dietary protein) as the main protein sources. Microbial phytase was added to the soybean meal based diet (1 000 and 2 000 IU·kg^{-1}) and to the fish meal based diet (2 000 IU·kg^{-1}) in order to study its effect on phosphorus utilisation. Results of this trial showed that growth rate, feed conversion and nitrogen retention were significantly better in fish fed the fish meal based diet. Energy retention was similar in both groups. ADC of protein were similar among groups. ADC of phosphorus was significantly higher in the fish meal based diet (63 %) than in the soybean meal based diet (25 %). Supplementation of the fish meal based diet with microbial phytase did not improve the ADC of phosphorus, while in the soybean meal based diet the inclusion of 1 000 and 2 000 IU·kg^{-1} of microbial phytase significantly increased the ADC of phosphorus to 71.5 % and 79.8 %, respectively. It is concluded that for practical purposes supplementation of diets with microbial phytase may prove valuable in diets including high levels of plant feedstuffs. © Ifremer/Elsevier, Paris

Microbial phytase / digestibility / phosphorus / *Dicentrarchus labrax* / seabass

Résumé – Utilisation d'aliments additionnés de phytase d'origine microbienne par des juvéniles du bar (*Dicentrarchus labrax*). Une expérience a été menée afin de comparer les performances de croissance, la composition corporelle, les coefficients de digestibilité apparente (ADC) de différents régimes alimentaires chez des bars d'un poids initial de 13,5 g, nourris d'aliments à base de farine de poisson (68,6 % des protéines alimentaires) ou de soja (65,6 % des protéines alimentaires) comme sources principales de protéines. Des phytases d'origine microbienne ont été ajoutées à chaque aliment afin d'étudier leurs actions sur l'utilisation du phosphore. Les résultats montrent que le taux de croissance, le taux de conversion alimentaire et la rétention azotée sont significativement meilleurs pour les poissons nourris avec les aliments contenant de la farine de poisson. La rétention d'énergie est identique chez les deux groupes, ainsi que l'ADC des protéines. L'ADC du phosphore est significativement plus élevé chez les poissons nourris avec les aliments contenant de la farine de poisson (63 %) que chez ceux nourris avec les aliments contenant du soja (25 %). L'addition de phytase d'origine microbienne aux aliments à base de poisson n'améliore pas l'ADC du phosphore, tandis que pour l'aliment à base de soja, l'ajout de 1 000 et 2 000 IU·kg^{-1} de phytases augmente de façon significative l'ADC du phosphore (71,5 et 79,8 % respectivement) On peut conclure que l'addition de phytase d'origine microbienne peut être intéressante lorsque les aliments contiennent une forte proportion d'ingrédients d'origine végétale. © Ifremer/Elsevier, Paris

Phytase microbienne / digestibilité / phosphore / *Dicentrarchus labrax* / bar

1. INTRODUCTION

Phosphorus, along with nitrogen, are the two main limiting nutrients for algal growth and, therefore, for water eutrophication. The discharge of these nutrients in fish farm effluents is directly or indirectly associated with feed and, in order to reduce water pollution and enhance a sustainable aquaculture development, these outputs must be minimised [3]. Nowadays, nutritionists and feed manufacturers are well aware of the importance of diets which satisfy, but do not exceed, phosphorus requirements of the species of interest [9, 15]. Accordingly, constraints are imposed on dietary phosphorus levels and, therefore, feed ingredients are selected taking into consideration both phosphorus content and phosphorus bioavailability.

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In spite of high world demand and increasing costs of fish meal, this feedstuff remains the main protein source in carnivorous fish diets [17, 27]. However, the need to reduce dietary phosphorus levels implies that at least part of the fish meal component is replaced by alternative protein sources. Contrary to animal feedstuffs, which usually have high phosphorus levels with a high bioavailability, plant feedstuffs have low phosphorus levels, and bioavailability of phosphorus is also low [18]. Between 60 % to 75 % of the phosphorus in plant-based feedstuffs is present in the form of phytate [21] which is indigestible in most monogastric vertebrates including finfish, as they lack the enzyme phytase [15].

In higher monogastric vertebrates, the inclusion of exogenous phytase to diets improves the bioavailability of phytate phosphorus [21]. In fish, similar results have been observed [4, 12, 23–25]. The inclusion of microbial phytase to diets containing high levels of plant proteins reduces the need for inorganic phosphorus supplements and hence contributes to a reduced discharge potential for phosphorus in fish farm effluents.

The aim of this trial was to study the effect of microbial phytase supplements in diets incorporating fish meal or soybean meal as the main protein sources upon growth performance and phosphorus utilisation by seabass juveniles.

2. MATERIAL AND METHODS

This experiment was performed at the Marine Station, at Porto, with seabass (*Dicentrarchus labrax*) juveniles obtained from a commercial hatchery. The study consisted of a growth and a digestibility trial.

Diets used were formulated to be isonitrogenous and isolipidic and to include fish meal (diet A) or soybean meal (diet B) as the main protein source (*table I*). Diets were not supplemented with inorganic phosphorus. Three other diets were also prepared: diet A1, which was identical to diet A but included 2 000 IU·kg^{-1} phytase, and diets B1 and B2, which were identical to diet B but included 2 000 or 1 000 IU·kg^{-1} phytase, respectively. The phytase used in this trial was obtained by fermentation from an *Aspergillus niger* strain (Natuphos® 5000, BASF, Germany). Dietary ingredients, after mixing thoroughly, were dry pelleted in a laboratory pellet mill (CPM).

The growth trial lasted 12 weeks and was carried out in a thermoregulated recirculation water system equipped with ten cylindrical fiberglass tanks of 100 L capacity. During the trial, water temperature was regulated to 20 °C and water salinity ranged between 32 and 34. Before starting the trial, the fish were acclimatised to the experimental facilities for two weeks. Thereafter, ten groups of 20 fish with an average individual weight of 13.5 g were assembled in each tank. Duplicate groups of fish were fed each experimental diet to satiation twice a day, except on the day before weighing. During the trial, fish were bulk weighed every two weeks, following a mild anesthesia (ethylene glycol monophenil ether: 0.3 mL·L^{-1}). At the start and at the end of the trial, eight fish from the stock population and five fish from each experimental tank, respectively, were killed and frozen at –20 °C for body composition analysis.

The digestibility trial was performed in a different thermoregulated recirculation water system, equipped with five 50-L fiberglass tanks with a faeces settling column connected to the outlet of each tank [5]. Five groups of 22 fish from the stock population were assembled in the tanks and acclimatised to the experimental conditions. Then, each group was fed twice a day to satiation one of the experimental diets for a 10-day period. The fish were allowed to acclimatise to the feed for the first 3 days, and faeces were collected over the next 7 days. Thereafter, the diets were randomly changed between groups, and the same procedure was repeated two more times. In each 10-day period, faeces from each tank were pooled and frozen at –20 °C until analysis, giving a total of three pooled faecal samples for each diet.

Table I. Composition of experimental diets.

Diets	A	A1	B	B1	B2
Ingredients (g·kg^{-1} DM)					
Fish meal[1]	450	450	160	160	160
CPSP[2]	50	50	50	50	50
Soybean meal[3]	154	154	580	580	580
Wheat[4]	260	260	90	90	90
Cod liver oil	50	50	84	84	84
Vitamin premix[5]	6	6	6	6	6
Mineral premix[6]	10	9.6	10	9.6	9.8
Choline chloride (50 %)	5	5	5	5	5
Lignin sulfate	10	10	10	10	10
Chromic oxide	5	5	5	5	5
Microbial phytase		0.4		0.4	0.2
Proximate analysis					
Dry matter (%)	88.0	89.0	87.5	87.2	91.7
Protein (N × 6.25; % DM)	48.5	47.6	47.3	47.0	47.8
Lipids (% DM)	13.4	13.3	13.5	13.2	12.8
Energy (kJ·g^{-1} DM)	21.0	18.9	19.9	21.5	20.9
Ash (% DM)	8.3	8.2	7.1	7.0	7.5
Phosphorus (% DM)	0.95	0.94	0.69	0.65	0.68
Chromic oxide (% DM)	0.42	0.50	0.53	0.50	0.45

[1] Standard fish meal from Norway (70 % protein), supplied by Sopropêche, France.
[2] Fish protein concentrate, supplied by Sopropêche, France.
[3] Toasted, dehulled, solvent extracted soybean meal, supplied by Sorgal, Portugal.
[4] infrared micronised.
[5] Vitamins: (IU·kg^{-1} diet): retinol, 18 000; cholecalciferol, 2 500; (mg·kg^{-1} diet): alpha tocoferol, 100; menadione bisulfite, 20; thiamin, 5; riboflavin, 5; Ca pantothenic acid, 40; nicotinic acid, 100; pyridoxine, 10; folic acid, 5; cyanocobalamin, 0.05; biotin, 0.5; ascorbic acid, 200; inositol, 300.
[6] Minerals (mg·kg^{-1} diet): potassium iodide, 16; sodium chloride, 6 000; copper sulfate, 50; iron sulfate, 126; manganese sulfate, 172; zinc sulfate, 288.

Table II. Growth and feed utilisation by seabass fed the experimental diets (mean ± SD; in the same row, numbers with different letters are significantly different, $P < 0.05$).

Diet	A	A1	B	B1	B2
Final weight (g)	32.9 ᵇ ± 1.8	34.2 ᵇ ± 0.4	27.3 ᵃ ± 0.4	29.4 ᵃ ± 0.4	28.6 ᵃ ± 0.4
Specific growth rate (%)[1]	0.99 ᵇ ± 0.06	1.04 ᵇ ± 0.02	0.78 ᵃ ± 0.02	0.86 ᵃ ± 0.02	0.84 ᵃ ± 0.01
Feed intake:weight gain	1.83 ᵃ ± 0.11	1.79 ᵃ ± 0.004	2.42 ᵇ ± 0.07	2.40 ᵇ ± 0.21	2.66 ᵇ ± 0.03
Weight gain:protein intake	1.28 ᵇ ± 0.08	1.32 ᵇ ± 0.003	1.00 ᵃ ± 0.03	1.02 ᵃ ± 0.09	0.86 ᵃ ± 0.01
N intake ($g \cdot kg^{-1}$ gain)	141.8 ᵃ ± 8.4	136.4 ᵃ ± 0.3	183.2 ᵇ ± 5.0	180.3 ᵇ ± 15.8	203.6 ᵇ ± 2.6
N retention (% N intake)	22.8 ᵇᶜ ± 1.7	23.1 ᶜ ± 0.1	16.5 ᵃ ± 0.9	17.7 ᵃᵇ ± 1.5	14.4 ᵃ ± 1.3
N retention ($g \cdot kg^{-1}$ gain)	32.2 ᵃ ± 0.5	31.5 ᵃ ± 0.0	30.2 ᵃ ± 0.9	31.8 ᵃ ± 0.0	29.2 ᵃ ± 2.3
Energy retention (% energy intake)	20.8 ᵃ ± 3.5	24.5 ᵃ ± 3.0	18.9 ᵃ ± 2.8	17.3 ᵃ ± 1.2	18.7 ᵃ ± 0.7

[1] Specific growth rate (%): (ln final weight – ln initial weight) × 100/n° days.

Analysis of diets, animals and faeces were undertaken as follows: dry matter after drying at 105 °C until constant weight; ash by burning in a muffle furnace at 450 °C for 16 h; lipids by petroleum ether extraction in a Soxhlet apparatus; protein (N × 6.25) by the Kjeldhal method, after acid hydrolysis; chromic oxide according to Furukawa and Tsukahara [6]; phosphorus by a spectrophotometric method using the vanado-molybdate method [19]; energy by direct combustion of samples in a micro-bomb calorimeter (Newham Electronics, model AH9).

Statistical evaluation of the data was done by analysis of variance (ANOVA) using a probability level of 0.05 for rejection of the null hypothesis. Differences among means were determined by applying Tukey's multiple range test.

3. RESULTS

At the end of the growth trial, final weight, feed conversion ratio and protein conversion ratio of fish fed the fish meal based diet (diet A) were significantly higher than that of fish fed the plant protein based diet (diet B) (*table II*). N intake ($g \cdot kg^{-1}$ gain) was significantly higher in fish fed diet B than in those fed diet A. N retention ($g \cdot kg^{-1}$ gain) was similar among groups, however, as a percentage of N intake, N retention was significantly higher in fish fed diet A than in those fed diet B. There were no differences in energy retention (% energy intake) between the two groups.

Regarding body composition of whole fish, at trial end there was an increase in lipid and in energy content and a decrease in ash and phosphorus content, relative to initial values. No significant differences in body composition among groups were observed at the end of the trial (*table III*).

Apparent digestibility coefficient (ADC) of dry matter of diet A was significantly higher than that of diet B (*table IV*). However, no differences were recorded between other diets for ADC of dry matter or between diets for any other examined parameter except for phosphorus of diet B, which was significantly lower than that of all other diets.

Phosphorus intake ($g \cdot kg^{-1}$ gain) was not significantly different among groups (*table V*). There were also no differences among groups in phosphorus retention, both as a percentage of phosphorus intake or per kg of gain. Regarding phosphorus excretion, the main differences between groups were the proportions of faecal and soluble P losses. Faecal losses ($g \cdot kg^{-1}$ gain) were two times higher in fish fed diet B than in fish fed diet A. The inclusion of phytase to the fish meal based diet (diet A1) had no effect on P utilisation. On the contrary, in the soybean meal based diets (diets B1 and B2) phytase inclusion significantly reduced faecal losses.

Table III. Body composition at the start and at the end of the trial of seabass fed the experimental diets (fresh weight basis).

Diet	Start	End				
		A	A1	B	B1	B2
Water (%)	70.7 ± 1.8	69.5 ± 0.4	68.1 ± 0.9	68.2 ± 0.3	68.4 ± 0.3	67.2 ± 0.9
Protein (%)	17.4 ± 0.1	17.5 ± 0.0	17.5 ± 0.0	17.0 ± 0.2	17.4 ± 0.0	17 ± 0.7
Lipids (%)	7.5 ± 1.2	11.3 ± 0.03	12.0 ± 1.1	11.3 ± 0.1	11.8 ± 1.0	12.3 ± 0.3
Energy ($kJ \cdot g^{-1}$)	4.9 ± 0.4	6.7 ± 0.5	7.0 ± 0.5	7.0 ± 0.5	7.0 ± 0.3	7.8 ± 0.4
Ash (%)	5.7 ± 0.6	3.9 ± 0.1	4.1 ± 0.2	4.4 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
Phosphorus (%)	0.75 ± 0.10	0.60 ± 0.03	0.61 ± 0.05	0.64 ± 0.03	0.66 ± 0.03	0.63 ± 0.03

Table IV. Apparent digestibility coefficients of the experimental diets (mean ± SD; in the same row, numbers with different letters are significantly different, $P < 0.05$).

	A	A1	B	B1	B2
Dry matter (%)	77.0 ᵇ ± 2.1	70.9 ᵃᵇ ± 4.1	66.1 ᵃ ± 3.4	71.5 ᵃᵇ ± 3.0	71.0 ᵃᵇ ± 3.8
Protein (%)	92.8 ᵃ ± 0.6	91.6 ᵃ ± 1.4	93.7 ᵃ ± 0.5	92.6 ᵃ ± 4.6	94.3 ᵃ ± 2.5
Phosphorus (%)	63.0 ᵇ ± 3.1	63.4 ᵇ ± 2.8	25.2 ᵃ ± 11.0	79.8 ᵇ ± 17.8	71.5 ᵇ ± 13.8

4. DISCUSSION

In this trial, growth performance of seabass fed diet A was similar to that observed in other studies with the

Table V. Phosphorus balance in seabass fed the experimental diets (mean ± SD; in the same row, numbers with different letters are significantly different, $P < 0.05$).

Diet	A	A1	B	B1	B2
Intake (g P·kg^{-1} gain)	17.4 ^{a} ± 1.0	16.8 ^{a} ± 0.0	16.7 ^{a} ± 0.5	15.6 ^{a} ± 1.4	18.1 ^{a} ± 0.2
Retention (% intake)	18.9 ^{a} ± 5.6	21.4 ^{a} ± 6.4	17.7 ^{a} ± 2.4	24.3 ^{a} ± 6.7	17.0 ^{a} ± 1.4
Faecal losses (% intake)	36.9 ^{a} ± 3.1	36.6 ^{a} ± 2.8	74.8 ^{b} ± 11.0	20.2 ^{a} ± 17.8	28.5 ^{a} ± 13.7
Soluble losses (% intake)	44.2 ^{b} ± 5.6	42.0 ^{b} ± 6.4	7.6 ^{a} ± 2.4	55.5 ^{b} ± 6.7	54.5 ^{b} ± 1.4
Retention (g P·kg^{-1} gain)	3.2 ^{a} ± 0.8	3.6 ^{a} ± 1.1	3.0 ^{a} ± 0.5	3.8 ^{a} ± 0.7	3.1 ^{a} ± 0.3
Faecal losses (g P·kg^{-1} gain)	6.4 ^{c} ± 0.4	6.2 ^{bc} ± 0.0	12.5 ^{d} ± 0.3	3.1 ^{a} ± 0.3	5.2 ^{b} ± 0.1
Soluble losses (g P·kg^{-1} gain)	7.7 ^{b} ± 1.4	7.1 ^{b} ± 1.1	1.3 ^{a} ± 0.4	8.7 ^{b} ± 1.8	9.9 ^{b} ± 0.1

same species, at identical water temperature [2, 10]. Compared to diet A, seabass fed diet B showed a significant reduction in growth rate and feed utilisation. In a previous trial [1], it was observed that growth of seabass fed diets including up to 44 % of protein from soybean was identical to that of seabass fed control fish meal based diet. Thereafter, the present results seem to indicate that higher incorporation levels of soybean protein (65.6 % of total protein) have negative effects on performance of seabass juveniles.

Several authors have evaluated soybean meal as a protein source to replace, partially or completely, fish meal in fish diets. Some studies demonstrate that soybean can totally replace fish meal in fish diets [14], however the majority of research demonstrates that high replacement levels of fish meal with soybean meal negatively affects fish performance [20]. Among other factors (unpalatability, anti-nutritional factors), this may be due to amino acid imbalance, since supplementation of diets incorporating high levels of soybean meal with limiting essential amino acids improves fish growth [14, 27].

Phytate present in soybean can also restrict its nutritive value, as it can form protein and mineral-phytic acid complexes, resulting in reduced protein and mineral availability [28]. It has been shown that rainbow trout (*Oncorhynchus mykiss*) fed purified diets including 0.5 % of phytic acid and common carp (*Cyprinus carpio*) fed purified diets including 0.5 or 1 % phytic acid had a significant reduction in growth and feed efficiency [11, 26]. In contrast, purified diets including up to 1.5 % of phytic acid had no adverse effect on growth or feed efficiency of channel catfish (*Ictalurus punctatus*) [7]. In the present study, comparison of the results obtained with diet B with those of diets B1 and B2 indicates that the reduction of the phytic acid content of the diets due to microbial phytase supplements had no significant effects on growth rate and feed efficiency.

In the present trial, phosphorus retention (g·kg^{-1} gain) of seabass was similar to values obtained in other fish species [8, 13, 24, 25]; although phosphorus content of whole fish (0.6–0.75 % fresh weight) was slightly higher than reported previously (0.4–0.5 %) [15].

Although phytic acid is expected to lower gastrointestinal absorption of proteins [15], this effect was not apparent in the present study as evidenced by ADC of protein which were not affected by increasing amounts of dietary soybean. Similar observations have been made previously [16, 22, 29]. As expected, ADC of phosphorus were significantly higher in fish meal based diet than in soybean meal based diet, and were within the values reported in salmonids for these feedstuffs [15, 24].

As previously reported in other species [4, 23–25] supplementation of diet B with microbial phytase significantly improved the ADC of phosphorus, and this effect was apparently higher than that observed in other studies. However, these comparisons should be regarded with care, as the faecal collection methods employed and the amount of phytase added to the diet may considerably affect the results [4, 12].

From the results of this study, it may be concluded that supplementation of practical diets for seabass, including high levels of plant feedstuffs with microbial phytase significantly improves phosphorus digestibility. Therefore, this will reduce the need for inorganic phosphorus supplements to diets, and will also reduce the phosphorus discharge in fish farm effluents.

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