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Replacement of fish meal with a matrix of organic plant proteins in organic trout (*Oncorhynchus mykiss*) feed, and the effects on nutrient utilization and fish performance

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ABSTRACT

This study examined the effects on nutrient utilization and fish performance when replacing 16, 31, and 47% of fish meal protein (corresponding to replacing 15, 29 and 44%, respectively, of total dietary protein) with a fixed matrix of organic pea, horsebean and rapeseed plant protein concentrates (PPC) in a ratio of 1.07:1.00:0.66. Four iso-energetic and iso-nitrogenous diets were produced to include 0, 136, 274 or 410 g kg^{-1} of the organic PPC matrix, respectively. The organic protein ingredients were chosen based on their high protein content, and the matrix was established to mirror the amino acid composition of fish meal. The plant ingredients were dried, dehulled, grinded and air classified in accordance with the European Union Commission Regulation on organic aquaculture production, increasing the protein concentrations up to 577 g kg^{-1} dry matter. Two experiments were carried out using juvenile rainbow trout (*Oncorhynchus mykiss*): 1) a digestibility study to examine the apparent digestibility of protein, lipid, nitrogen-free extract (NFE), total phosphorus and phytate-phosphorus, followed by a water sampling period to determine the output of nitrogen and phosphorus and enabling the setup of nitrogen and phosphorus mass-balances; and 2) a 57 day growth study including 3 growth periods each of 19 days and using pit-tagged fish. Substituting fish meal with organic PPC significantly increased the apparent digestibility coefficient (ADC) of protein and lipid ($P<0.008$) at the highest PPC inclusion level, while there was a significant ($P<0.044$) decrease in the ADC of NFE with increasing PPC inclusion level. The apparent digestibility coefficient of phytate-phosphorus was significantly lower ($P<0.005$) at the highest PPC inclusion level compared to the fish meal control diet. The mass-balances revealed a significant increase in the excretion of ammonium-nitrogen (NH_4N, $P<0.017$) at the two highest PPC inclusion levels and a decrease in phosphorus ($P<0.009$) excretion at the highest organic PPC inclusion level. There was no overall effect on the specific growth rates (SGRs) or feed conversion ratios (FCRs). The study thus demonstrated that it is possible to replace fish meal by 47% organic PPC without compromising rainbow trout performance. However, the results also indicated that it will be difficult to replace much more than this as long as supplementation with synthetic amino acids and exogenous phytase is not allowed in organic feed.

1. Introduction

The principles of organic aquaculture encourage the development of fish feeds containing fish meal from sustainable fisheries only to avoid depleting global fish stocks (EU, 2007, 2009). In combination with an increasing demand for organic trout (Bergleiter et al., 2009), this stresses the need for alternative, organic feed ingredients.

Only a couple of previous studies have examined the effects of replacing fish meal with organic protein ingredients on fish performance, and none of the studies have looked at rainbow trout. Lunger et al. (2006, 2007) found that up to 40% fish meal protein may be replaced by organically certifiable protein sources in feed for juvenile cobia (*Rachycentron canadum*) without negatively affecting performance.

In contrast to organic feed, much of the current research in conventional (i.e. non-organic) feed for salmonids examines the substitution of fish meal by vegetable proteins (e.g., Gatlin et al., 2007; Glencross et al., 2010; Øverland et al., 2009), and studies have shown that it is possible to substitute a significant part of fish meal with plant protein concentrates without compromising fish growth when supplementing the diet with indispensable amino acids (e.g., Kaushik et al., 1995; Rodehutscord et al., 1995). Such results cannot be directly applied to organic aquaculture where the organic code of

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practice implies certain limits to the feed (reviewed by Mente et al., 2011). Following the European Union's regulation No. 834/2007 on organic production and labeling of organic products (EU, 2007), it is not allowed to add synthetic amino acids to the feed or to use chemically solvent related purification methods of the plant ingredients. It is therefore necessary, when formulating an organic diet, to blend a selection of different vegetable protein sources with high protein contents and complementary amino acid profiles, since optimization of the amino acid profile of organic feed must be based on the protein sources alone. An optimized amino acid profile can only be obtained by combining a number of plant protein ingredients as no single agricultural crop can provide a suitable amino acid composition (Gaylord et al., 2010; Kaushik, 1990).

Proteins in high quality fish meal are palatable, highly digestible (~90%) and anti-nutritional factors (ANFs) are more or less non-existing (Gatlin et al., 2007; Gaylord et al., 2010). Substituting fish meal with organic plant ingredients thus faces further challenges, as plant-based ingredients often contain a variety of indigestible carbohydrates some of which may also have anti-nutrient effects, along with a number of non-carbohydrate anti-nutrients (Francis et al., 2001; Jezierny et al., 2010; Krogdahl et al., 2010). The latter includes phytate (myo-inositol hexaphosphate), which is the main phosphorus storage form in plants (Cheryan, 1980; Ravindran et al., 1994). Phytate-phosphorus is highly unavailable to carnivorous fish, which lack the enzyme phytase needed for catalyzing the hydrolysis of phytate and rendering the phosphorus available for uptake (Ellestad et al., 2002; Pallauf and Rimbach, 1997; Sajjadi and Carter, 2004). As processing any feed materials with the aid of chemically synthesized solvents or supplementation with exogenous phytase is not allowed in feed for organic trout, the availability of dietary phosphorus may become a limiting factor for organic fish.

Another consequence of the restrictions against chemically synthesized solvents is that it is difficult to reduce the content of indigestible carbohydrates in organic plant feed ingredients, which have been shown to reduce the nutritional value of conventional feed in many fish species (e.g., Glencross, 2009; Krogdahl et al., 2010; Refstie et al., 1999).

The objective of the present study was to examine the effects on nutrient utilization and fish performance when gradually substituting fish meal by a matrix of organic pea (*Pisum sativum*) protein concentrate (PC), organic horsebean (*Vicia faba*) protein concentrate (HC), and organic rapeseed (*Brassica napus*) protein concentrate (RS). The three plant protein sources were chosen based on their, for plant protein ingredients, relatively high protein content, and the matrix was established to mirror the amino acid composition of high quality fish meal.

2. Materials and methods

2.1. Protein sources and diet composition

Danish produced organic pea beans, organic horse beans and organic rapeseed were obtained from Toft Foods A/S, DLF-TRIFOLIUM A/S and Lehnsgaard Aakirkeby respectively, while a high quality, low temperature (LT) fish meal was obtained from FF Skagen, Denmark. The plant protein sources were dried, dehulled, grinded, and air classified at the Centre of Process Innovation, Technological Institute, Denmark, to reduce the content of anti-nutrients and obtain crude protein concentrations of 512, 518 and 331 g kg^{-1} in the pea, horse bean, and rapeseed meal, respectively.

Four iso-energetic and iso-nitrogenous experimental diets (A, B, C, D) were formulated by BioMar Ltd based on proximate analyses of the four protein feed ingredients (Table 1). Diet A served as a control diet containing fish meal as the primary protein source (i.e., fish meal constituting 94% of total dietary protein), while 16, 31 and 47% of the fish meal protein in diet A (corresponding to 15, 29 and 44%, respectively, of total dietary protein) were replaced by an organic protein matrix consisting of PC, HB and RS in the ratio 1.07:1.00:0.66 (Table 1) in diet B, C, and D, respectively. The maximum inclusion level of the plant protein concentrate (PPC) matrix (i.e., 44% of total dietary protein) was determined by the protein content and amino acid composition of the PPC matrix. Wheat was used as filler to balance the diets.

The diets were produced by the Danish Technological Institute using a twin-screw Werner & Pfleider 37 extruder and fabricated as 3.0 mm pellets. They were stored at 2 °C throughout the study.

The crude protein and lipid content of the four experimental diets was quite similar, ranging between 44.2–46.0% for protein and 29.0–30.8% for lipid (Table 1). The total phosphorus (TP) content of the four diets was also very similar (1.43–1.47%), while the phytate-P content increased with increasing plant protein supplementation that is, increasing from 8.5% of TP in diet A to 21.5% of TP in diet D. High TP levels in the PPC diets were due to unexpectedly high levels of TP in the analyzed PPC batches deviating from common literature values.

There was generally little variation between the four diets in the content of essential amino acids except for methionine and threonine whose content decreased with increasing PPC inclusion (Table 1).

2.2. Experimental design and procedures

Two experiments were carried out: 1) A digestibility trial followed by a water sampling period to determine the apparent digestibility coefficients (ADCs) of dietary nutrients as well as the composition and magnitude of dissolved nitrogen (N) and phosphorus (P) waste produced, which enabled the construction of complete N and P budgets; and 2) a growth study to determine the specific growth rates (SGRs) and feed conversion ratios (FCRs) of the four diets. The experiments were carried out at the North Sea Research Centre, Denmark, using juvenile rainbow trout (*Oncorhynchus mykiss*) obtained from Binderup Fish Farm, Denmark.

2.2.1. Digestibility and mass-balance study (experiment 1)

This experiment lasted 24 feeding days and was designed as a fully random, single factorial experiment with three replicate tanks for each of the four experimental diets (i.e., $n = 3$ experimental units per diet, 12 tanks in all). Fish with an initial mean weight of 67.0 ± 7.3 g were randomly distributed at a stocking density of 19 fish tank^{-1} among twelve, 189 L, cylindrical–conical, flow-through, thermoplastic tanks in a modified Guelph setup as previously described (Dalsgaard and Pedersen, 2011). All fecal particles were collected in separated sedimentation columns submerged in ice-water to prevent biological breakdown between samplings. The tanks were supplied with 10 °C tap water at a flow rate of 40 L h^{-1}. A 15 h light:9 h dark diurnal photoperiod was maintained throughout the trial, and oxygen saturation levels were kept between 70 and 100% at all times. The fish were acclimatized to the experimental conditions and to the diets for 7 days prior to the commencement of the experiment. They were individually weighed at the start of the experiment (day 0), and a pooled sample of 9 fish was collected from each dietary treatment group serving as initial carcass samples, while all remaining fish in each tank were sacrificed by the end of the experiment, serving as final carcass samples.

The fish were fed 1.5% of their biomass d^{-1} for 12 days (calculated based on an expected FCR). The daily ration was divided into two equal portions which were fed at 10:00 and 14:00 h, respectively. Feed waste was registered and counted throughout the trial. All feces from the sedimentation columns were collected daily prior to feeding at 10:00 h, and samples from each three consecutive days were pooled (i.e., yielding four fecal sampling periods) and stored at −20 °C until chemical analysis was carried out. Feces from the first sampling period served as back-up samples, while feces from the second and third sampling periods were analyzed for protein,