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Replacement of dietary soy- with air classified faba bean protein concentrate alters the hepatic transcriptome in Atlantic salmon (*Salmo salar*) parr☆

Christian De Santis [a,*], Viv O. Crampton [b], Beatrix Bicskei [a], Douglas R. Tocher [a]

[a] Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
[b] EWOS Innovation, 4335 Dirdal, Norway

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ABSTRACT

The production of carnivorous fish such as Atlantic salmon (*Salmo salar*) is dependent on the availability of high quality proteins for feed formulations. For a number of nutritional, strategic and economic reasons, the use of plant proteins has steadily increased over the years, however a major limitation is associated with the presence of anti-nutritional factors and the nutritional profile of the protein concentrate. Investigating novel raw materials involves understanding the physiological consequences associated with the dietary inclusion of protein concentrates. The primary aim of the present study was to assess the metabolic response of salmon to increasing inclusion of air-classified faba bean protein concentrate (BPC) in feeds as a replacement for soy protein concentrate (SPC). Specifically, we tested treatments with identical contents of fishmeal (222.4 g kg^{-1}) and progressively higher inclusion of BPC (0 g kg^{-1}, 111.8 g kg^{-1}, 223.6 g kg^{-1}, 335.4 g kg^{-1}, 447.2 g kg^{-1}) substituting SPC. This study demonstrated a dose-dependent metabolic response to a plant ingredient and was the first to compare the nutrigenomic transcriptional responses after substitution of terrestrial feed ingredients such as BPC and SPC without withdrawal of marine ingredients. It was found that after eight weeks a major physiological response in liver was only evident above 335.4 g kg^{-1} BPC and included decreased expression of metabolic pathways, and increased expression of genes regulating transcription and translation processes and the innate immune response. Furthermore, we showed that the nutritional stress caused by BPC resembled, at least at hepatic transcriptional level, that caused by soybean meal (included as a positive control in our experimental design). The outcomes of the present study suggested that Atlantic salmon parr might efficiently utilize moderate substitution of dietary SPC with BPC, with the optimum inclusion level being around 120 g kg^{-1}in the type of feeds tested here.

1. Introduction

The sustainable and profitable production of farmed fish is increasingly dependent on their capacity to perform under dietary regimes based on plant ingredients. Carnivorous fish species such as Atlantic salmon (*Salmo salar*) are in high demand due in part to their beneficial effects on human health as well as the eating quality of their flesh (Whelton et al., 2004, Johnston et al., 2006). To satisfy the increasing demand, high quality protein sources are required for the formulation of the feeds. The stagnation of wild fisheries and the limited availability of marine products such as fishmeal (FM) (FAO 2008–2015), traditionally the ideal protein source for farmed fish (National Research Council, 2011), has increased pressure for the introduction of alternative raw materials as dietary source of proteins (Gatlin et al., 2007). In recent years, considerable research has addressed the performance of fish utilizing alternative feed materials partially or completely substituting FM. Thus far, plant meals and proteins such as soy have been the most economically viable alternative raw materials (Gatlin et al., 2007). As a result of ongoing research, modern commercial feeds for Atlantic salmon utilize significant inclusion levels of alcohol-extracted soy protein concentrate (SPC) as the predominant substitute for FM with, to a lesser extent, a range of other plant sources (Ytrestøyl et al., 2014). However, the use of SPC as the major alternative to FM has raised economic (price of soy fluctuates due to high demand from a number of industries), environmental (over-exploitation of land for farming), strategic (over-reliance on a single ingredient for feed manufacture) and nutritional (less balanced composition compared with mixed sources) concerns, prompting continuing research towards the development of new alternative raw materials to be used in combination with others as protein concentrate (Burr et al., 2012, Zhang et al., 2012, De Santis et al., 2015a).

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* Corresponding author at: Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK. Tel.: +44 1786 467993.
E-mail address: Christian.desantis@stir.ac.uk (C. De Santis).

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To be nutritionally attractive for commercial use, candidate alternative feedstuffs for salmon feeds must have relatively high protein content (48%–80%, ideally higher than 60%) with a reasonable balance of essential amino acids, and have low levels of carbohydrates (e.g. fibre and starch) and antinutritional factors (ANFs) (Gatlin et al., 2007). In addition, good alternative feed ingredients must be accessible in terms of availability, price and sustainability. Many of these characteristics are partially dependent on the processing of the raw material that can greatly affect the resulting feedstuff both nutritionally and economically. In this respect, protein concentrates from faba bean (*Vicia faba*) (BPC) possess a number of favourable characteristics. Recently, we investigated the performance of Atlantic salmon fed BPC produced by air-classification, a simple and inexpensive process (De Santis et al., 2015a). Using a mixture model approach the effects of 16 different combinations of FM, SPC and BPC on growth and health performance of salmon were assessed using parr in freshwater. The screening study aimed to identify an appropriate and more confined range of replacement levels to be applied in seawater studies involving larger fish. It was demonstrated that salmon efficiently utilized BPC at inclusion levels ranging from 50 to 200 g kg^{-1}, partially replacing SPC and/or FM, resulting in increased growth, protein content, fat content and ash. Evidence of detrimental effects on gut health, commonly observed in response to dietary inclusion of some vegetable proteins such as soybean meal (SBM) (Baeverfjord and Krogdahl, 1996, Urán et al., 2008 and Kortner et al., 2012), were not observed at low levels of inclusions (De Santis et al., 2015a). In contrast, it was shown that a high inclusion level of BPC (447.2 g kg^{-1}) caused mild gut inflammation, comparable but not as severe as that caused by SBM in post-smolt, seawater adapted salmon. The most important outcome of that study was that a superior performance was observed in response to mixed ingredients with the optimum formulation being 200.8 g kg^{-1} FM, 268.9 g kg^{-1} SPC, and 117.4 g kg^{-1} BPC, providing a strong basis for continuing research on BPC utilization in salmon (De Santis et al., 2015a).

Nutrigenomics is a powerful approach to determine detailed metabolic responses (Mutch et al., 2005). Recently, nutrigenomics has been used as a tool to study the response of fish to vegetable dietary proteins, primarily focusing on hepatic or intestinal profiles (Panserat et al., 2009, Skugor et al., 2011, Kortner et al., 2012, Overturf et al., 2012, Tacchi et al., 2012 and De Santis et al., 2015b). Specifically in salmon, two studies have reported the hepatic transcriptional signatures underlying a SBM-induced nutritional stress (Skugor et al., 2011 and De Santis et al., 2015b). Further studies are however required to elucidate, understand and discriminate the general and specific molecular mechanisms underlying utilization of terrestrial proteins in salmon and fish in general. In this context, the present study aimed to provide insights into the metabolic responses of salmon parr to the utilization of air classified BPC as an alternative source of dietary protein.

The overall aim of the present study was to determine and compare hepatic transcriptomes in Atlantic salmon fed increasing levels of BPC as a substitute for dietary SPC. It is important to emphasize the rationale behind the feeds tested in this study. The experimental feeds used (B0, B20, B40, B60, B80) contained the same level of FM and varying levels of two vegetable proteins: SPC, a refined protein concentrate obtained by aqueous alcohol extraction of soybean, widely established as a dietary ingredient of farmed Atlantic salmon (Ytrestøyl et al., 2014) and BPC, a protein concentrate from faba bean produced with the a dry processing method (air-classification). In addition, a feed formulated with high levels of SBM (360 g kg^{-1}) was included as positive control to benchmark detrimental effects associated with the plant material and affected by the processing method. Specifically, the objectives of this study were to *a*) establish if the mild effects on gut metabolism, health and impaired growth observed after high inclusion of BPC (i.e. 447.2 g kg^{-1}) was reflected in the alteration of hepatic metabolism, perhaps similar to that observed with high inclusion of SBM (positive control for nutritional stress); *b*) provide metabolic evidence to determine the maximum level of BPC inclusion that is efficiently utilized by salmon; and *c*) understand the metabolic processes underlying the improved growth performance observed previously with low/moderate BPC inclusion (De Santis et al., 2015a) by studying the response to lower inclusion levels of BPC (i.e. 111.8 g kg^{-1}, 223.6 g kg^{-1}). A well-described and validated custom-made Atlantic salmon 44 K oligo microarray was utilized for the nutrigenomic profiling. The present study demonstrated a dose-dependent metabolic response to a plant ingredient and represents the first report in fish where the transcriptional response to three terrestrial feed ingredients (BPC, SPC and SBM) is compared.

2. Materials and methods

2.1. Nutritional trial and experimental treatments

The nutritional trial, including full experimental design and diet formulations is described in detail elsewhere (De Santis et al., 2015a). Briefly, the trial was conducted in the freshwater facilities of EWOS Innovation (Dirdal, Norway) using a farmed population of Atlantic salmon parr of average initial weight of around 1.5 g. Fish were acclimatized for two weeks before application of the experimental feeds, which were then fed to quadruplicate tanks. All feeds were formulated to meet the nutritional requirement of salmon (National Research Council, 2011) and to have the same protein, lipid and energy content. After eight weeks of feeding, liver was dissected from 24 individuals per dietary treatment (6 per tank), immediately placed in RNA Later (Life Technologies, Paisley, UK) and processed as per the manufacturer's instructions before being stored at −20 °C prior to analyses. For hepatic transcriptional profiling a subset of five dietary treatments was chosen to span the most heterogeneous range of growth and health performance and allow to directly comparing SPC and BPC. The treatments had identical contents of FM (222.4 g kg^{-1}) and progressively higher inclusion of BPC (0 g kg^{-1}, 111.8 g kg^{-1}, 223.6 g kg^{-1}, 335.4 g kg^{-1}, 447.2 g kg^{-1}) substituting SPC referred to as diets B0, B20, B40, B60, and B80 respectively (Table 1). In addition, a feed formulated with 440 g kg^{-1} FM and 360 g kg^{-1} SBM was also analysed as a positive reference. Since knowledge on the hepatic transcriptomic response of Atlantic salmon parr to plant proteins is limited, we used the positive control to benchmark and define transcriptional profiles that could be supposedly associated with detrimental effects of the plant. Inclusion levels of SBM similar to those used in this study are in fact well documented to induce enteropathy in adult salmon (Baeverfjord and Krogdahl, 1996, Urán et al., 2008, Urán et al., 2009, Krogdahl et al., 2010). The feeds analysed in this study corresponded to 20:80:00 (B0), 20:40:40 (B20), 20:60:20 (B40), 20:20:80 (B60), 20:00:80 (B80) and HiSBM (SBM) from our previous nutritional trial (De Santis et al., 2015a) and were renamed for clarity of presentation and understanding.

2.2. Transcriptome analysis

Transcriptomic analysis was conducted using custom-made 4 × 44 K Atlantic salmon oligo microarray slides (Agilent Technologies,

Table 1
Formulations of experimental feeds.

Ingredients	B0	B20	B40	B60	B80
LT FM	222.4	222.4	222.4	222.4	222.4
Selecta SPC 60	448.2	336.2	224.1	112.1	0.00
Fabaqua 62–65	0.00	111.8	223.6	335.4	447.2
Wheat gluten	80.0	80.0	80.0	80.0	80.0
Tapioca	76.1	76.3	76.6	76.7	77.0
Vitamin, mineral and pigments premixes	56.6	56.6	56.6	56.6	56.6
Synthetic amino acids	8.3	9.1	10.0	10.7	11.5
Fish oil	98.4	97.6	96.8	96.1	95.3
Lecithin source	10.0	10.0	10.0	10.0	10.0

Formulation of the experimental feeds used in the study. All values are represented as g kg^{-1}. Further details on dietary formulations can be found in De Santis et al. (2015a).