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Atlantic salmon (*Salmo salar*) parr as a model to predict the optimum inclusion of air classified faba bean protein concentrate in feeds for seawater salmon

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ABSTRACT

Faba bean (*Vicia faba*) is a legume with good potential that has previously been tested in fish species with some promising results. The present study aimed to determine whether an air-classified faba bean protein concentrate (BPC, 615 g kg^{-1} crude protein content) could offer improved or favourable growth performance, body composition and gut health compared to commercially used protein sources such as FM and soy protein concentrate (SPC) in Atlantic salmon (*Salmo salar*). The trial investigated the performance of 16 feeds formulated with varying FM/SPC/BPC proportions using a mixture design approach. Salmon parr of average weight of 1.47 g were used as a model. The trial lasted eight weeks and also included high FM (560 g kg^{-1}) and high defatted soybean meal (SBM, 360 g kg^{-1}) feeds as negative and positive controls respectively, for the assessment of enteritis in the distal intestine. The effects on growth performance, body nutrient composition, survival and fish health, specifically gut histology, were determined. The results demonstrated conclusively that total inclusion levels of BPC ranging from 50 to 200 g kg^{-1}, partially replacing SPC and/or FM, displayed the greatest potential to be beneficial in terms of fish performance and nutrient composition with increased growth, protein content, fat content and ash. In addition to favourable whole-body composition parameters, it was found that inclusions of BPC below 340 g kg^{-1} of feed did not cause detrimental effects such as the enteritis observed in fish fed the high soybean meal control. High inclusion level (447.2 g kg^{-1}) of BPC caused a mild inflammation that was not as severe as that caused by the feed with high SBM. The results of this screening study indicate that BPC derived from faba beans can be a valuable alternative protein source in Atlantic salmon feeds. The data provided a platform to model the optimum range of BPC inclusion levels in combination with FM and SPC for further investigation in commercially relevant fish and conditions.

1. Introduction

Plant products with high protein content and amino acid compositions that meets the nutritional requirement of fish are sought after (Gatlin et al., 2007). Soy protein concentrate (SPC) processed from defatted, alcohol washed soybeans has proved to be a source of protein that is efficiently utilized by carnivorous fish such as salmonids (Glencross et al., 2005). Along with good nutritional profiles, SPC has the advantage, unlike many other plant protein concentrates, of being available in sufficient volumes to fulfil the rising demand and allow a high level of inclusion in commercial European salmonid and marine fish feeds. As a result, modern commercially available feeds for species such as Atlantic salmon (*Salmo salar*) often contain 200–250 g kg^{-1} SPC as a dominating substitute of FM, meaning that feed manufacturers heavily rely upon this plant product as a source of replacement (Ytrestøyl et al., 2014).

Heavy reliance upon a single ingredient can have important nutritional and commercial implications. Nutritionally, the abundant use of a single vegetable/plant alternative as a replacement to FM can translate into higher concentration of specific types of anti-nutritional factors (ANFs) that potentially exceeds the level physiologically tolerable by the fish. Several adverse effects have been reported in fish fed plant-derived ANFs, including amongst others reduced nutrient digestibility, pancreatic hyperactivity, inflammation of the distal intestine and impaired bone mineralization (Baeverfjord and Krogdahl, 1996; Denstadli et al., 2006; Gu et al., 2014; Knudsen et al., 2007; Kortner et al., 2012; Storebakken et al., 2000). It is well known that different plant products may vary in content and composition of ANFs, with unrefined or defatted soybean meal containing arguably the highest levels (Francis et al., 2001). In this respect, using a combination of plant protein sources might increase the number of ANF species in the feed while reducing the

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concentration of each individual ANF. This may enable fish to cope better with lower levels of a mixture of ANFs rather than higher levels of any individual factor. In addition to biological/nutritional benefits, being able to rely upon multiple raw materials might translate into greater flexibility when formulating the feeds, into increased purchasing power and also into mitigating the negative effects of any undesirable between-batch differences upon final feed quality. All these characteristics are highly desirable from a commercial perspective. For the above-mentioned reasons it is critically important to investigate new and improved raw materials with high potential to be used as a protein source in fish feeds.

Faba bean (*Vicia faba* L.) also known as field bean, horse bean or broad bean is a legume with good potential as an ingredient for inclusion in salmon feeds. Faba bean crops are grown in a variety of climates including both in Northern Europe and North America (FAO STAT) and they can be successfully used in crop rotation to help reduce the use of nitrogen fertilisers made from fossil fuel sources. A significant proportion of the ANF present in faba beans are concentrated in the seed coat, which is removed in the process of de-hulling (Vidal-Valverde et al., 1998). Meals from whole or de-hulled faba beans (FBM) have been investigated as replacement for FM or soybean meal (SBM) in feeds for omnivorous and carnivorous fish species such as Nile tilapia (*Oreochromis niloticus*) and rainbow trout (*Oncorhynchus mykiss*) fingerlings with promising results (Azaza et al., 2009; Gaber, 2006; Ouraji et al., 2013). One important limitation of FBM, however, is the relatively low protein concentration (~30%) that does not allow a formulation that easily fulfils the total dietary protein required in modern commercial feeds for carnivorous species. To overcome this limitation, additional processing is required in order to obtain a product with higher protein concentration.

The process of fine-grinding and air-classification has been utilized previously to produce a protein concentrate from faba beans (herein termed bean protein concentrate, BPC) with a highly desirable profile (i.e. 55–60% protein content) for inclusion in commercial salmon feeds (Gunawardena et al., 2010). The advantage of the air classification technology is that there is no requirement for solvents or acids, thus making it a relatively simple and low-cost process. Such simplicity also means that ANFs are not likely to be degraded, but may co-purify with proteins and possibly be present in higher concentration in the final BPC compared with the original de-hulled material. Despite the potential advantages associated with the use of faba beans in combination with air-classification technology, there have been no reported studies assessing the applicability of this product, individually or in combination with other plant protein products, as an ingredient for Atlantic salmon feeds. In contrast, air classification was applied to another legume, the field pea (*Pisum sativum*), and tested in salmon feeds. Promising results were reported when inclusions of pea protein concentrates (PPC) with up to 50% crude proteins were moderate (i.e. 200 g kg^{-1}) but higher levels (350 g kg^{-1}) in combination with other plant products induced inflammation of the distal intestine (Øverland et al., 2009; Penn et al., 2011).

The present study was the first to provide experimental evidence of efficient utilization of air classified BPC in Atlantic salmon. In particular, this research sought to determine whether mixtures of different protein sources including air classified BPC, SPC and FM could offer improved growth and favourable physiological responses compared to feeds formulated using only FM and SPC. In particular, and as a first step before working with seawater fish, a screening trial, using salmon parr, investigating the performance of 16 experimental feeds and two controls was performed. The primary aim of this study was to identify the optimal range of BPC inclusion levels that could be later tested in conditions more relevant to a commercial environment. Small fish were used as a model justified by the dramatic reduction of costs that allowed a large number of feeds to be tested in parallel and not for a specific evaluation of the life-stage requirements. The feeding trial utilized a mixture design approach which allowed a structured investigation of varying proportions of blends of FM, BPC and SPC.

2. Materials and methods

2.1. Ingredients, design and feed composition

In this experiment, feeds were designed using a mixture design approach with three factors. This experimental design was indicated as the most appropriate strategy to maximize the information on interactions between feed components (Ruohonen and Kettunen, 2004) and was previously utilized in fish (Zhang et al., 2012). The main advantage of the mixture design approach is the possibility to empirically predict the response to any combination of the blend, exclude those with the least promising potential and focus only on those displaying sub-optimal performances. To apply this particular experimental design, feeds required to be formulated using a variable and a fixed component. The fixed component (constituting 350 g kg^{-1} of the feed) was the same for all feeds and not expected to affect the observed response, while the variable component (constituting 650 g kg^{-1} of the feed) included the raw materials under examination (Table 1). The fixed component comprised FM Norse LT-94 (120 g kg^{-1}), wheat gluten (80 g kg^{-1}), tapioca (63 g kg^{-1}), fish oil (62 g kg^{-1}), mineral, vitamin and pigment premixes (25 g kg^{-1}) (the source of each ingredient is reported in Tables 1 & 2). In each blend of the mixture design, the proportion of each raw material summed to 100% (or 1000 g kg^{-1}). Due to the different composition of the raw materials (Table 1), three analogues or pre-mixes were formulated containing either FM, SPC or BPC along with other materials to allow them to be substituted for each other without altering the nutrient composition of the final feeds. Analogues were given the abbreviations FM_a, SPC_a and BPC_a, respectively (Table 2). From this point forward the suffix "a" is used to distinguish the analogue from the raw material on which it was based. The analogues were formulated to have identical digestible proteins, digestible energy, and the amino acids lysine, methionine + cystine and threonine (Table 3). Thus, since both SPC and BPC had a low concentration of some indispensable amino acids (especially methionine) compared to that of FM, synthetic amino acids were also added. Tapioca meal and mono calcium phosphate (MCP) were used as diluents. MCP was added to the SPC_a and BPC_a as a source of available phosphorus. Due to the higher energy content of the FM_a compared to SPC_a and BPC_a, it was necessary to add a diluent to the FM_a and a starch source (tapioca) and MCP were chosen for this purpose. The independent variable used for statistical modelling of the 16 experimental feeds was the mix of analogues used in each of the feeds and expressed as a proportion of the total variable component.

For processing, dry raw materials were mixed and grinded on Sprout Matador hammer mill fitted with a 0.75 mm screen. The meal mix was extruded on a Wenger Tx-57 twin screw extruder fitted with a Wenger

Table 1
Composition in g kg^{-1} of the fishmeal, soya and bean protein concentrates used in the experimental feeds.

	FM[a]	SPC[b]	BPC[c]
Crude protein	705.9	627.8	615.8
Crude fat	95	29	27
Starch	–	6.7	3.3
Selected amino acid composition			
Lysine	53.3	38.5	40.4
Methionine	19.1	8.3	4.2
Cystine	6.5	8.8	7.8
Threonine	58.7	24.6	21.8
Tryptophan	6.7	7.2	4.3

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