



Effects of different blends of protein sources as alternatives to dietary fishmeal on growth performance and body lipid composition of Atlantic salmon (*Salmo salar* L.)

J. Pratoomyot^{a,*}, E.Å. Bendiksen^b, P.J. Campbell^b, K.J. Jauncey^a, J.G. Bell^a, D.R. Tocher^a

^a Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK

^b BioMar AS, Nordregt. 11, N-7484 Trondheim, Norway

ARTICLE INFO

Article history:

Received 23 December 2010
Received in revised form 1 March 2011
Accepted 3 March 2011
Available online 21 March 2011

Keywords:

Fishmeal
Plant proteins
Regression analysis
Growth performance
Composition
Atlantic salmon

ABSTRACT

Recently, we reported that growth of Atlantic salmon was reduced as dietary fishmeal (FM) was lowered from 25% to 5% in dual-substituted feeds compared to a control diet, formulated to represent the current upper levels of substitution of FM and fish oil. In the present study, the effects of different alternative protein blends and binders on growth of salmon fed dual-substituted feeds containing only 11% FM, and with 60% of dietary fish oil replaced by rapeseed oil were investigated. Salmon of initial weight 1.3 kg were grown to market size (>3 kg) over a period of 19 weeks. Salmon fed the diets with reduced FM showed lower final weight, SGR and TGC, associated with reduced feed intake. There was a tendency for increased FCR in fish fed the diets containing reduced FM although this was not significant, and there was no effect on PER. There were no significant effects on digestibility of protein or fat but the two parameters varied reciprocally and there were clear trends of increased protein and lower fat digestibilities in fish fed diets with reduced FM. Although lipid and fatty acid compositions did not vary greatly between diets there were significant effects on fish tissue compositions. Thus, liver lipid was generally reduced in fish fed diets with lower FM, significantly so in two of the four treatments. The proportions of monoenes were significantly lower and those of polyunsaturated fatty acids (PUFA) significantly higher in flesh and liver of fish fed diets with reduced levels of FM. The increased proportions of PUFA were due to increased percentages of 20:4n-6, 20:5n-3, 22:5n-3 and, although not consistently significant, 22:6n-3. The mechanisms for these unexpected effects of diet on tissue lipids and fatty acids are discussed.

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1. Introduction

The supply of marine raw materials such as fishmeal (FM) and fish oil (FO), the predominant sources of protein and lipid for carnivorous fish feeds, has become a limiting factor for expansion of aquaculture due to the pressure on feed-grade fisheries (Naylor et al., 2009). Production of these marine raw materials cannot increase above current levels and, coupled with the increasing demand driving prices upwards, it is no longer feasible to use FM and FO at the current inclusion levels (FAO, 2009). This has led to the investigation of new, cost-efficient protein and oil sources as alternatives to FM and FO in aquafeeds (Hardy, 2010; Turchini et al., 2009). Previous studies indicated that replacement of FM and FO with protein and lipid sources from terrestrial plant and animal sources would be possible provided amino acid and fatty acid requirements are met (Glencross et al., 2007; Webster et al., 2007).

Several land animal products including poultry by-products, meat, bone and blood meals have been investigated as substitutes for FM in fish diets (Smith et al., 1995; Robaina et al., 1997; Webster et al., 1999). However, plant products potentially offer more sustainable protein sources for aquafeeds although they often contain anti-nutritional factors, which can affect growth performance and fish health (Francis et al., 2001; Gatlin et al., 2007). Thus rapeseed, soybean and sunflower meals as well as various legumes (beans and peas) are less expensive and readily available in high quantities, although they have variable desirable and undesirable characteristics that both support and limit their use (Francis et al., 2001; Gill et al., 2006; Krogdahl et al., 2010). Corn gluten meal has been shown to be a good alternative for FM replacement in salmon feeds, being low in anti-nutritional factors (Mente et al., 2003), and wheat gluten has high digestibility and palatability although prices tend to fluctuate due to production restrictions and demand (Hardy, 1996). Soy protein concentrates (SPC) have been reported as good sources for partial substitution of FM for many species of fish including salmon without reducing growth (Refstie et al., 2001), although problems with gut damage have been reported at inclusion >20% of some soybean products in salmon (Baeverfjord and Krogdahl, 1996; Knudsen et al.,

* Corresponding author. Tel.: +44 1786 467993; fax: +44 1786 472133.
E-mail address: jarunan.pratoomyot@stir.ac.uk (J. Pratoomyot).

2007). Legumes including field peas and pea protein have been shown to be good protein sources for Atlantic salmon (Aslaksen et al., 2007).

Partial replacement of FM with plant meals at a variety of different levels of substitution has been studied in several fish species including salmonids (Kaushik et al., 1995; Espe et al., 2006; Torstensen et al., 2008). Generally, the replacement of up to 30–40% FM with single plant meals does not compromise growth of fish (Nengas et al., 1996; Refstie et al., 2001; Lozano et al., 2007). However, replacement of greater than 70% of dietary FM by blends of plant meal resulted in negative effects on growth of various fish including salmonids (Gomes et al., 1995; Espe et al., 2006; Torstensen et al., 2008). Although complete replacement of FM by plant meals has generally not been very successful, substitution of close to 100% of dietary FM by blends of plant proteins was possible in salmonids with no major negative effects on growth if the amino acid profile in the feed was well balanced, and if feed intake was comparable to a high FM diet (Barrows et al., 2007; Espe et al., 2007). This demonstrated the potential of replacing dietary FM with mixtures of alternative protein sources in Atlantic salmon.

In a recent study, we reported the effects of progressive reduction in dietary FM in dual-substituted feeds for Atlantic salmon. Compared to a control diet, formulated to represent the current upper levels of substitution of FM and FO, growth of Atlantic salmon was progressively reduced as the FM content of the diet was reduced from 25% to 5% (Pratoomyot et al., 2010). In the present study, we tested the hypothesis that the negative effects on growth were due to factors associated with the specific protein replacers used and that alternative combinations of replacers could avoid these effects. Therefore, different alternative protein blends (mixtures of sunflower meal, corn gluten meal, soybean meal, wheat gluten, pea protein and blood meal) and binders (faba and kidney beans) were investigated in salmon fed dual-substituted feeds with almost 90% of FM replaced and 60% of dietary FO replaced by rapeseed oil. Atlantic salmon of initial weight of 1.3 kg were grown to market size (>3 kg) on the different feeds over a period of 19 weeks and the effects on growth performance, feed utilization

efficiency, protein and fat digestibility, and lipid and fatty acid compositions of flesh and liver investigated.

2. Materials and methods

2.1. Diets and animals

Five diets were formulated to satisfy the nutritional requirements of salmonid fish (NRC, 1993) and manufactured at BioMar Tech-Centre, Brande, Denmark. All diets contained 35% crude protein and 28% crude lipid. The control diet was formulated to contain 25% FM, the minimum level of FM inclusion currently used in commercial salmon culture, and the other experimental diets contained 11% FM. The protein ingredients of the control diet (25F) contained 25% FM and 45% alternative proteins (a blend of plant proteins including sunflower meal, corn gluten, soybean protein concentrate and faba bean). The basal ingredients of the other four experimental diets were 11% FM and 55% alternative proteins that were, diet 11FW (qualitatively similar blend to 25F plus wheat gluten), 11FP (qualitatively similar blend to 11FW plus pea protein), 11FB (qualitatively similar blend to 11FW plus blood meal) and 11FK (qualitatively similar blend to 11FW plus kidney bean). All diets were coated with a 60:40 blend of rapeseed oil and northern hemisphere FO and all the experimental diets were supplemented with crystalline indispensable amino acids, lecithin and carophyll pink to meet requirement levels for amino acids, phospholipid and pigments (Table 1).

Two thousand two hundred and fifty Atlantic salmon of initial mean weight 1.3 ± 0.1 kg were randomly distributed among 15 cages of 125 m^3 with 150 fish/cage at the Marine Harvest Fish Trials Unit, Ardnish, Scotland. The fish were fed one of five diets in triplicate cages. The experiment was conducted over 19 weeks from October 2007 to February 2008 under natural photoperiod with average water temperature ranging from 7 to 11 °C. Fish were fed to apparent satiation by automatic feeders (Sterner Arvo-tec UK, Inverness,

Table 1
Feed formulation (mg kg^{-1}) and proximate composition (%) of the diets.

Feed ingredients	25F	11FW	11FP	11FB	11FK
Fishmeals ¹	250	110	110	110	110
Sunflower expeller	115	40	4	108	40
Corn gluten	85	175	130	100	175
Soy concentrate ²	85	175	130	100	175
Wheat gluten	–	18	17	11	18
Pea protein	–	–	130	–	–
Haemoglobin meal	–	–	–	84	–
Field beans	160	160	160	160	–
Kidney beans	–	–	–	–	160
Rapeseed oil ³	173	178	176	180	178
Fish oil ⁴	116	118	117	120	118
Micronutrients ⁵	11.95	23.59	25.48	21.49	23.59
Crystalline amino acids ⁶	1.19	5.43	4.88	2.32	5.43
Lecithin	5.0	5.0	5.0	5.0	5.0
Astaxanthin ⁷	0.40	0.40	0.40	0.40	0.40
Antioxidant ⁸	4.25	4.25	4.25	4.25	4.25
Proximate composition ⁹					
Protein	34.3 ± 0.4^c	35.0 ± 0.1^{bc}	34.5 ± 0.3^b	36.1 ± 0.2^a	35.3 ± 0.1^b
Lipid	29.8 ± 0.1^a	27.9 ± 0.1^c	27.5 ± 0.3^c	28.8 ± 0.3^{bd}	28.3 ± 0.3^{cd}
Moisture	6.7 ± 0.1^a	6.2 ± 0.0^b	6.2 ± 0.1^b	6.5 ± 0.1^a	5.5 ± 0.1^c
Ash	6.0 ± 0.1^a	5.2 ± 0.1^{bc}	5.4 ± 0.1^b	5.0 ± 0.0^c	5.3 ± 0.1^b
NFE ¹⁰	23.2 ± 0.3^b	25.7 ± 0.2^a	26.4 ± 0.3^a	23.6 ± 0.2^b	25.6 ± 0.3^a

¹ Peruvian fishmeals produced from Anchoveta.

² Soy protein concentrate (SPC60; 60% crude protein) produced from defatted soy flakes.

³ Non-GM double-low rapeseed oil.

⁴ North-Atlantic standard fish oil.

⁵ Vitamin and mineral premixes with limestone carrier added according to the commercial standards of BioMar AS.

⁶ Highly purified (99%) crystalline amino acids.

⁷ Carophyll pink CWS 10%.

⁸ Blend of antioxidants and starch carrier added according to the commercial standards of BioMar.

⁹ Results are means \pm SD ($n = 3$). Values within a row with different superscript letters are significantly different as determined by ANOVA.

¹⁰ NFE (nitrogen free extract) calculated by subtraction, $100 - (\text{crude protein} + \text{crude fat} + \text{moisture} + \text{ash})$.

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