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Effect of stachyose, raffinose and soya-saponins supplementation on nutrient digestibility, digestive enzymes, gut morphology and growth performance in Atlantic salmon (*Salmo salar*, L)

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ABSTRACT

This study investigated the effects of raffinose, stachyose either alone or in combination, and the role of soya-saponins in combination with these oligosaccharides, in diets for Atlantic salmon on nutrient digestibilities, growth performance and morphological changes of the gastrointestinal tract. The experiment was carried out using triplicate groups of Atlantic salmon with 0.421 kg initial weight and lasted for 68 days. The experimental diets consisted of a negative control diet based on high-quality fish meal (FM), and a positive control diet containing 300 g kg^{-1} soybean meal (SBM), and four diets based on the negative control diet added 6.7 g kg^{-1} raffinose (RA), 29.5 g kg^{-1} stachyose (ST), a combination of the raffinose and stachyose (RA–ST), or the same combination further supplemented with 2 g kg^{-1} soya-saponins (RA–ST–SA). The results showed that fish fed the SBM, the positive control, had significantly higher FCR, and tended to have lower weight gain and growth rate compared to fish fed the FM-diet, while fish fed the RA, ST, RA–ST and RA–ST–SA ranked in between the two control diets. The results indicate that feeding the Atlantic salmon the RA, ST, RA–ST and RA–ST–SA did not interfere with protein or fat digestibility. None of the dietary treatments, except for the positive control, caused significant morphological changes in the liver, or mid and distal intestines.

1. Introduction

The alcohol soluble fraction in soybean meal contains bioactive compounds shown to cause morphological changes (van den Ingh et al., 1991, 1996; Knudsen et al., 2007; Yamamoto et al., 2008) in the distal intestine of several fish species such as rainbow trout (Bureau et al., 1998; Heikkinen et al., 2006; Romarheim et al., 2008; Yamamoto et al., 2008), Atlantic salmon (Krogdahl et al., 2003; Baeverfjord and Krogdahl, 1996; Refstie et al., 2000, 2005), Atlantic cod (Olsen et al., 2007) as well as common carp (Uran et al., 2008). Soya-saponins have in some recent publications (Knudsen et al., 2007, 2008) been associated with the onset of morphological changes known as soybean meal induced enteritis in Atlantic salmon. Knudsen et al. (2008) demonstrated that soya-saponins added to a fishmeal based diet caused no morphological changes, whereas enteritis was observed when the soya-saponins were added to a diet with 25% lupin kernel meal. Iwashita et al. (2009) showed that soya-saponins in combination with lectin caused abnormal histology in the distal intestine of rainbow trout. However, not all indicators of soybean induced enteritis were observed. These studies suggest that soya-saponins in combination with one or several other components present in some legumes cause the morphological changes associated with soybean meal induced enteritis.

Signs typical of soybean induced enteritis in the distal intestine have also been observed in Atlantic salmon fed diets with 35% peas (Penn et al., 2011). Soybean meal, lupins and peas have in common that they all contain saponins and oligosaccharides (Bach-Knudsen, 1997; Storebakken et al., 2000; Martinez-Villaluenga et al., 2005; Rochfort and Panozzo, 2007). The main oligosaccharides found in soybeans are the galacto-oligosaccharides raffinose, stachyose, and verbascose. Because the nutritional value of soy and lupin protein concentrates (Van den Ingh et al., 1996; Glencross et al., 2003), and fermented soy (Refstie et al., 2005) – all with reduced oligosaccharide content – is higher than that of the whole products, it has been suggested that oligosaccharides are involved in the negative effects on nutrient digestibility and feed utilization in salmonids. However, most other studies investigating the effects of oligosaccharides have used soybean meal (Refstie et al., 2005), lupins (Glencross et al.,

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2003) or alcohol-soluble extracts containing oligosaccharides and saponins (Van den Ingh et al., 1996; Knudsen et al., 2007, 2008; Yamamoto et al., 2008). It has, therefore, not been possible to conclude whether raffinose, stachyose or verbascose alone, or in combination with other antinutrients such as soya-saponins (Knudsen et al., 2008) lead to reduced nutrient utilization and enteritis.

The content of oligosaccharides in soybeans is variable (Hartwig et al., 1997; Grieshop et al., 2003; Hollung et al., 2005, 2006), but raffinose and stachyose are the most predominant. In general, legumes contain more stachyose than raffinose (Kuo et al., 1988; Karr-Lilienthal et al., 2005; Hollung et al., 2005, 2006). Although galacto-oligosaccharides in soybean meal are not expected to be a main causative factor for enteritis in SBM fed fish (Van den Ingh et al., 1991), they may contribute to the observed reduction in fecal dry matter content and possibly affect the microflora (Olli and Krogdahl, 1995a,b; Refstie et al., 1999). Moreover, galacto-oligosaccharides in feed for Atlantic salmon have been found to reduce protein retention (Grisdale-Helland et al., 2008). More information is warranted regarding the effects of raffinose and stachyose in feed for Atlantic salmon.

Saponins are naturally occurring glycosides in legumes, and saponins from different plant sources have different biological activities due to their large structural diversity. Saponins from different plants have been associated with reduced feed intake due to bitterness (Cheeke, 1996) and morphological changes in fish as in the case of soya-saponins (Bureau et al., 1998; Knudsen et al., 2007, 2008). Knudsen et al. (2008) showed that inclusion of a partially purified preparation of soya-saponins in salmon diets caused increased gut permeability and suggested that saponins in plant ingredients may impair the barrier function of the intestinal epithelium making the underlying mucosa more exposed to antigens from micro flora or proteins from the lumen. Some positive effects of saponins have also been reported. *Quillaja* saponins are reported to have a growth promoting effect in Nile tilapia and common carp (Francis et al., 2001a, b, 2002, 2005), though the validity of these results have been challenged (Gatlin et al., 2007). It has been reported elsewhere that soya-saponins slightly increase growth in Atlantic salmon, depending on the protein source of the diet (Venold et al., 2010). Though some information regarding the biological activity of soya-saponins in fish exists, the subject has not been extensively studied.

The main objective of the present study was to investigate the effects of raffinose and stachyose alone or in combination, with and without soya-saponins, in diets for Atlantic salmon on nutrient digestibility, growth performance and morphological changes in the gastrointestinal tract.

2. Materials and methods

2.1. Production of extruded diets

Diet formulation and analyzed chemical composition of the diets are shown in Table 1. Prior to diet formulation, the concentrations of stachyose and raffinose in the soybean meal (SBM) and the purity of raffinose and stachyose products were analyzed. The content of stachyose and raffinose in the SBM chosen for the experiment was 26.06 mg kg DM^{-1} and 6.67 mg kg^{-1} DM, respectively. A fish meal based diet (FM) and SBM-based diet (SBM) were formulated as negative and positive controls, respectively. The other experimental diets were formulated from the FM diet supplemented with either raffinose (RA), or stachyose (ST), or a combination of these two sugars (RA–ST), or these two sugars in combination with soya-saponins (RA–ST–SA). The supplemented diets contained about 3 times more stachyose and raffinose compared to the SBM diet. The level of saponins was chosen to approximate the amount of saponins in the SBM diet. Yttrium oxide (Y_2O_3) was used as an inert marker (Austreng et al., 2000).

Six diets were extruded using a 2.5 mm die to obtain 3 mm feed particles. The feeds were produced at Ewos Innovation AS, Dirdal, Norway. The ingredients were milled with a Matador (Jesma, Vejle, Denmark) 650/315 hammer-mill and sieved through a 1.25 × 1.25 mm mesh, mixed for 4 min in a Sprout Matador (Jesma) HPB500 paddle mixer, conditioned for approximately 1.5–2 min in a Wenger (Sabetha, KS, USA) differential diameter conditioner (DDC) and extruded in a Wenger X-85 single screw extruder with a length:diameter ratio of 13.1:1. The feeds were produced using the same extrusion processing parameters and they were produced continuously when steady state

Table 1
Ingredient and chemical composition of the experimental diets (g kg^{-1}).

	Control (FM)	Soybean meal (SBM)	Raffinose (RA)	Stachyose (ST)	Raffinose + stachyose (RA–ST)	Raffinose + stachyose + saponins (RA–ST–SA)
Fishmeal[a]	739.9	417.9	733.2	710.4	703.7	701.7
Wheat[b]	120.0	120.0	120.0	120.0	120.0	120.0
Soybean meal[c]		300.0				
Fishoil[d]	115.0	137.0	115.0	115.0	115.0	115.0
Wheat gluten[e]	20.0	20.0	20.0	20.0	20.0	20.0
Micro ingredients[f]	5.0	5.0	5.0	5.0	5.0	5.0
Stachyose[g]				29.5	29.5	29.5
Raffinose[h]			6.7		6.7	6.7
Saponins[i]						2.0
Yttriumoxide[j]	0.1	0.1	0.1	0.1	0.1	0.1
Dry matter, g kg^{-1}	965.3	934.3	919.2	903.8	915.7	922.5
In dry matter, kg^{-1}						
Protein, g	600.9	541.8	594.2	575.3	570.4	573.4
Fat, g	168.6	153.5	185.2	183.2	178.4	180.0
Ash, g	100.1	81.2	99.2	95.3	97.5	95.3

[a] Norse® LT-94, low-temperature dried fish meal, Egersund Sildeoljefabrikk AS, Egersund, Norway.
[b] Food grade, FK Agri, Askim, Norway.
[c] Deno-soy F®, with hulls, hexane extracted, toasted, Denofa, Fredrikstad, Norway.
[d] Fish oil, Egersund Sildeoljefabrikk AS, Egersund, Norway.
[e] Vital wheat gluten, Roquette, Lestrem Cedex, France.
[f] Commercial blend, Ewos Innovation, Dirdal, Norway.
[g] Stachyose (79% purity) produced by China National Research Institute of Food and Fermentation Industries, Bejing, China.
[h] Raffinose (99% purity) produced by Shanghai Nuotai Chemical Co., LTD, Shanghai, China.
[i] Soyasaponin concentrate (95% purity) produced by Organic Technologies, Coshocton, OH, USA.
[j] Metall Rare Earth Limited, Guojishizhang Jiaoliuzhongxin, Shenzhen, China.