Aquaculture 298 (2009) 101–110

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

An assessment of organ and intestinal histomorphology and cellular stress response in Atlantic salmon (*Salmo salar* L.) fed genetically modified Roundup Ready® soy

N.H. Sissener [a,*], A.M. Bakke [b,c], J. Gu [b], M.H. Penn [b,c], E. Eie [a,d], Å. Krogdahl [b,c], M. Sanden [a], G.I. Hemre [a]

[a] *National Institute of Seafood and Nutrition Research (NIFES), Bergen, Norway*
[b] *Norwegian School of Veterinary Science, Oslo, Norway*
[c] *Aquaculture Protein Centre, a CoE, Norway*
[d] *Skretting ARC, Stavanger, Norway*

ARTICLE INFO

Article history:
Received 14 August 2009
Received in revised form 7 October 2009
Accepted 12 October 2009

Keywords:
Atlantic salmon
Genetic modification
Roundup Ready® soy
Stress
Intestine

ABSTRACT

This study was conducted to investigate potential differences between genetically modified (GM) Roundup Ready® soy and its near-isogenic maternal line as feed ingredients for Atlantic salmon, with focus on intestinal changes commonly caused by soybean meal, histomorphology of other organs and stress response. A 7-month feeding trial was conducted with an inclusion level of 25% GM soy in the diet. Samples for histology were collected after 4 months, after 6 months, when a cross-over of the diet groups was conducted, and at the end of the trial of the crossed-over groups. Histomorphology of spleen, head kidney and mid intestine exhibited no differences between the diet groups, while glycogen deposits in liver were decreased in the GM fed fish at the final sampling. Common soybean meal-induced changes of the distal intestine in Atlantic salmon were observed in both diet groups at all sampling points, within levels expected at the current inclusion level of soy in the diets. However, mucosal fold height in the distal intestine was lower in the GM fed group at one of the three sampling points, and mucosal fold fusion was more pronounced in this group overall in the trial. A stress test conducted at the end of the trial gave responses in haematological parameters, plasma nutrients and mRNA transcription of heat shock protein (HSP) 27 in both liver and distal intestine, but responses were similar between the two diet groups, indicating similar ability to handle stress. The cross-over design, implemented to look at reversibility of potential GM-effects, proved to be inadequate as the crossing of diet groups in itself caused responses that would obscure possible minor diet effects. In conclusion, minor differences were observed between the diet groups; however, GM soy did not appear to cause any adverse effects on organ morphology or stress response compared to non-GM soy.

1. Introduction

The potential occurrence of unintended effects of genetic modification is one of the issues to be addressed in safety assessment of genetically modified (GM) plants used as feed and food (Kuiper and Kleter, 2003). Transgene insertion is an imprecise and poorly understood event, and introduction of superfluous DNA, as well as deletions and rearrangements of host DNA at the insertion site, are common occurrences (Somers and Makarevitch, 2004; Latham et al., 2006). This might disrupt transcription of endogenous genes, resulting in unintended changes in levels of macro- or micronutrients, anti-nutritional factors (ANFs) or production of toxic compounds (Cellini et al., 2004). The regulatory process is designed to look for these types of changes in GM plants, but this is a targeted approach that will never cover all known and unknown compounds in the plant. At present, the EU has approved about 30 GM plant products for use in foods and feeds (http://ec.europa.eu/food/dyna/gm_register/index_en.cfm). However, knowledge regarding possible health effects in animals and man is still sparse (Pryme and Lembcke, 2003; Domingo, 2007). Most studies conducted with GM plants have been relatively short term and focused on production parameters such as growth, milk production or meat yield, rather than investigating early biomarkers for physiology, health and reproduction parameters. Discussions regarding the necessity of evaluating products apparently similar to traditional counterparts (EFSA, 2008), and lack of standardized methods for the evaluation of unintended effects (Kuiper and Kleter, 2003), might be reasons for limited research activity in this area.

Processed soybeans are the largest source of protein feed and the second largest source of vegetable oil in the world. Of the global

Abbreviations: ALAT, alalanine aminotransferase; ASAT, aspartate aminotransferase; ANF, anti-nutritional factor; Ct, threshold cycle; FFSBM, fullfat soybean meal; GM, genetically modified; Hct, haematocrit; Hb, haemoglobin; HSP, heat shock protein; LDH, lactate dehydrogenase; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume; MF, mitotic figures; MFH, mucosal fold height; MMC, melanomacrophage centre; PCR, polymerase chain reaction; RBC, red blood cell; RRS®, Roundup Ready® soy; SBM, soybean meal; TAG, triacylglycerol.

* Corresponding author. NIFES, Postboks 2029 Nordnes 5817 Bergen, Norway. Tel.: +47 41462385; fax: +47 55905290.

0044-8486/$ – see front matter
doi:10.1016/j.aquaculture.2009.10.011

acreage planted with soybeans, 64% is now GM (James, 2007). The dominating GM variety is Roundup Ready® soy (RRS®), which is modified to be tolerant to the herbicide glyphosate (Padgette et al., 1995). Soy is commonly used in feed for Atlantic salmon at low levels and has, as a plant protein, a well balanced amino acid profile compared to the requirements of fish (Gatlin et al., 2007). However, the levels of standard qualities used in diets for Atlantic salmon are limited due to immunological responses in the intestine which seem to be dose and time dependent (Uran, 2008).

Effects of soybean meal (SBM) on the distal intestine of salmon include inflammatory responses (enteritis), which seem to be caused by one or several alcohol-soluble ANFs, such as saponins, phytosterols, oligosaccharides and/or other unidentified components (Van den Ingh et al., 1991; Baeverfjord and Krogdahl, 1996; Van den Ingh et al., 1996). Further, there are decreases in both in total weight and mucosal fold height, while lamina propria is widened and infiltrated by a mixed population of leukocyte cells (Van den Ingh et al., 1991; Baeverfjord and Krogdahl, 1996; Nordrum et al., 2000). As salmon are so sensitive to soy inclusion in the diet, even compared to other fish species (Evans et al., 2005), unintended alterations in GM varieties could have implications for the suitability of GM soy as a feed ingredient for this species.

In addition to the gastrointestinal tract, which is the first site of exposure to diet ingredients, the liver is a target organ in dietary studies. The liver is a key metabolic organ and has an important role in response to toxicants and immune response (Morin et al., 1993; Benninghoff and Williams, 2008; Tintos et al., 2008). Histomorphological changes have been observed in hepatocytes of mice fed GM soy, involving nuclear modifications that were shown to be reversible when mice were switched to a non-GM soy diet (Malatesta et al., 2002, 2005). Nuclear modifications of hepatocytes have also been observed in sheep fed Bt-maize (Trabalza-Marinucci et al., 2008). Furthermore, histological evaluations of other organs have been used to assess effects of GM ingredients in diets for mice and rats (Malatesta et al., 2003; Vecchio et al., 2004; Séralini et al., 2007). However, some of these studies have met criticism, and there are other feeding trials in which no differences between animals fed conventional or GM ingredients have been detected (Flachowsky et al., 2007).

Inadequate nutrition or harmful substances might only have an effect when conditions are suboptimal, as the fish will then struggle to maintain homeostasis. Thus, it might be interesting to compare stress response in fish fed non-GM and GM soy. The physiological stress response entails increases in stress hormones followed by activation of metabolic pathways, such as mobilization of energy reserves to maintain or attempt to re-establish homeostasis, and physiological responses such as alterations in blood chemistry and haematology (Barton and Iwama, 1991). On a cellular level, heat shock proteins (HSPs) have been proposed as an indicator of stressed states in fish (Iwama et al., 2004). Heat shock proteins are a highly conserved group of proteins found in a wide range of organisms from bacteria to humans (Morimoto et al., 1990, 1992; Welch, 1993; Feder and Hofmann, 1999), including fish (reviewed in Iwarma et al., 1998; Basu et al., 2002). With the exception of those of low molecular weight, such as HSP27, these proteins have constitutive functions in the unstressed cell (Morimoto et al., 1990; Hendrick and Hartl, 1993; Welch, 1993; Fink and Goto, 1998). However, various heat shock proteins are up-regulated in response to a wide variety of stressors, as they have a role in repair and degradation of misfolded or denatured proteins (Welch, 1993; Freeman et al., 1999; Rabergh et al., 2000). Increased levels of HSP70 have been observed in salmon fed soy as a replacement for fishmeal (Bakke-McKellep et al., 2007; Sagstad et al., 2008).

The aim of the current study was to assess whether GM RRS® soy affects organ histomorphology and cellular stress response in Atlantic salmon differently than near-isogenic non-GM soy, focusing on commonly observed SBM-induced effects in salmon. This work completes the evaluation of a 7-month feeding trial, where assessments of growth, body composition, organ sizes, haematology, plasma chemistry, lysozyme levels and performance through the parr-smolt transformation (Sissener et al., 2009) and liver proteome analysis (Sissener et al., in press), have been conducted to compare RRS® GM soy and its near-isogenic line as diet ingredients for Atlantic salmon.

2. Materials and methods

2.1. Experimental design and sampling

The 7-month feeding trial was conducted at the Institute of Marine Research (Matredal, Norway), and was approved by the National Animal Research Authority in Norway. Atlantic salmon with an initial average weight of 39.7 g (SD 4.4) were fed two different diets, with four replicate tanks of 120 fish for each diet group. Roundup Ready® soy was used in one diet (GM) and its near-isogenic, non-modified maternal line in the other (nGM). Both lines of soybeans were supplied by the Monsanto Company, St. Louis, MO, USA. The diets were compositionally similar in major nutrients (Table 1), and in both diets full fat soybean meal (FFSBM) provided 21% of the total protein. Further information on feed analysis, growth data, light regime and other details regarding fish husbandry is given elsewhere (Sissener et al., 2009). The feeding trial was initiated the 11th of August, and for the work presented in this paper samples were collected 13th of December (sampling 1), 2nd of February (sampling 2) and 28th of February (sampling 3). The fish were transferred from freshwater to seawater the day after sampling 1, as they were going through the parr-smolt transformation, which is a natural part of the salmon life cycle. Histological evaluation of the mid and distal intestine, spleen, head kidney and liver before seawater transfer (sampling 1) are

Table 1
Full fat soybean meal (FFSBM) composition, formulation and proximate composition of the diets.

	nGM	GM
FFSBM composition		
Protein (%)	36.7	38.5
Lipids (%)	22.6	20.4
Starch (%)	1.8	1.6
Ash (%)	4.8	5.1
Dry matter (%)	92.6	92.9
Residue (%)[1]	26.7	27.3
Formulation g kg $^{-1(2)}$		
Fishmeal	510	510
nGM soy	262	
GM soy		250
Fish oil	150	150
Soy oil		8
Wheat	75	79
Vitamin/mineral mix	3	3
Feed composition		
Dry matter (%)	94.0	93.8
Total protein (%)	46.1	45.8
Lipids (%)	24.2	24.6
Ash (%)	9.8	9.9
Starch (%)	6.1	6.2
Residue (%)[1]	7.9	7.3
Vitamin B6 (mg/kg)	14.7	14.8
Gross energy (kJ/g)[3]	21.5	21.6

[1]Residue was calculated as dry matter-(protein + lipid + starch + ash). [2]The fishmeal used was Norse-LT 94 Nordic fishmeal, made from 65% blue whiting, 30% sprat and 5% cuttoffs (fish industry byproducts) (Fiskernes Fiskeindustri, Denmark). Whole soybeans (RRS® and non-GM near-isogenic maternal line) were kindly supplied by Monsanto and ground to FFSBM by Skretting ARC. Wheat was bought from Dansk Landbrugs Grovvareselskap and the Vitamin/mineral mix from Trouw Nutrition (the Netherlands). [3]Gross energy was calculated according to (Tacon, 1987) using the energy content of 39.5 kJg^{-1} for lipid, 23.6 for protein and 17.2 for starch. The results are presented as the average of two analytical parallels.