EFFECT OF BILE PRECURSOR LEVEL AND NSP LEVEL ON FAT DIGESTIBILITY AND FAECAL LOSS OF BILE SALTS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Introduction

Unfortunately, for the majority of carnivorous fish, the digestive system is not built to handle large amounts of plant ingredients. Inclusion of such ingredients in the diet was shown to cause poor nutrient digestion, including of fat, and sometimes even caused occurrence of liver abnormalities (e.g., fatty liver, green liver syndrome) (Gu et al., 2013). Reduction in fat digestibility could be overcome by addition of exogenous bovine bile salts (Krogdahl et al., 2003). Furthermore, bile salt supplementation improved intestinal abnormality in some fish fed a plant-based diet (Yamamoto et al., 2007). These beneficial roles of bile salt supplementation highlight the urgency for a better understanding of the interactions between the bile metabolism in fish and diet composition when shifting from animal-based to plant-based ingredients.

The main idea behind this experiment is that “the bile salt metabolism is a limiting factor for fat digestion in carnivorous fish fed plant-based diets”. This limitation is expected to have a dual cause. On one side, using plant ingredients can put a strain on the bile salt metabolism trough increased fecal bile salt loss. In rats, soluble NSP’s have been shown to increase the excretion of bile salts via the feces by increasing the viscosity, thus decreasing their reabsorption efficiency (Ide et al., 1989). Furthermore, some NSP’s can bind bile salt, lipids and cholesterol (Vanhouny et al., 1981). On the other hand, a strain on the bile salt metabolism might occur because plant ingredients usually supply less of bile salt precursors (cholesterol, methionine and taurine), which are needed for endogenous synthesis. Apart from dietary intake, teleost fish have some capacity for de novo synthesis of taurine and cholesterol, but indications are that particular rate limiting enzymes might make this insufficient (Deng et al., 2012; El-Sayed, 2013). These limitations lead to a drop of the total bile salt pool that is present in the entero-hepatic circulation, possibly resulting in the observed decrease in fat digestion and gut/liver health in fish fed plant based diets. This experiment aimed to investigate whether increasing levels of NSP’s have a negative effect on fat digestibility and the bile metabolism via increased loss of bile salts. Furthermore, it was hypothesised that this effect is dependent on the dietary level of bile salt precursors.

Materials and methods

Six iso-lipidic diets were formulated according to a 2 X 3 factorial design. The first factor was level of bile salt precursors (taurine and cholesterol), which were altered by inclusion of either fish meal or a plant protein mixture as the main protein source. Fish meal-based diets were considered to have ‘high’ bile salt precursor levels, while the plant-based diets were considered to have ‘low’ levels. The second factor in the design was level of NSP’s. Within each group of precursor level, 3 diets were formulated by exchanging the main carbohydrate source with varying levels of a mixture of NSP rich ingredients. Levels of inclusion of that mixture were 0, 8 and 16% of final feed weight. The main fat source and

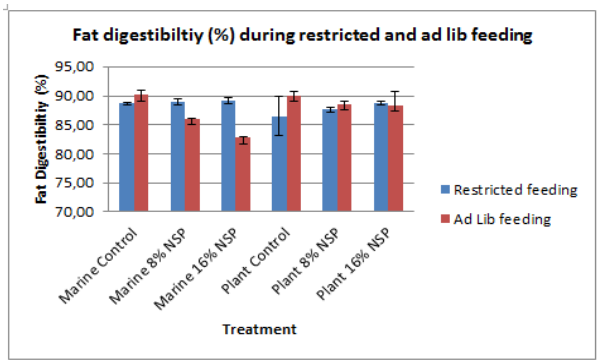
level of inclusion was kept constant in all diets. The last factor was feeding level. Fish were restrictedly fed one of the six diets for 4 week followed by ad lib feeding with the same diet for 3 weeks. Rainbow trout juveniles were randomly distributed into 12 experimental units (flow-through systems). Each unit was randomly assigned to one of the six dietary treatments in duplicate. The fish were hand fed twice a day restrictively for a period of 4 weeks, followed by ad lib feeding for 3 weeks. Fish were batch weighed at the beginning and end of each feeding period and faeces were collected daily and pooled per week. Tanks (n=12; treatment in duplicate) were used as experimental unit. A two way ANOVA (+ repeated measures to include feeding level) was used to check for effects. In case of a significant effects, a Tukey test was carried out for pairwise comparison of treatments. Statistical significance was tested at the 0.05 probability level. All data were tested for homogeneity of variance by Levene's test prior to ANOVA. Normal distribution of residuals was checked using the Kolmogorov-Smirnov test.

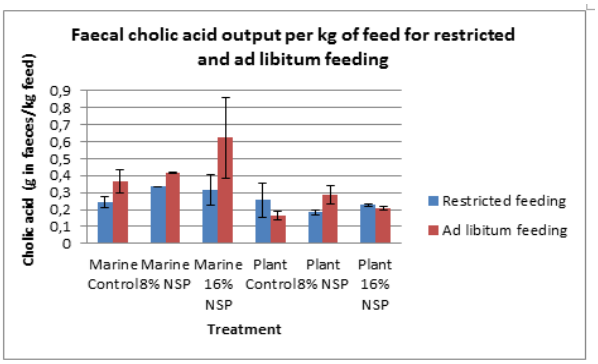
Results

At restricted feeding (1.5% BW/day), data showed no significant main effects (of both precursor and NSP level) on fat digestibility and faecal loss of bile salts. There was also no significant interaction effect.

In contrast, ad lib feeding did show significant effects on both fat digestibility and faecal bile salt loss. However, during interpretation of the data, it is important to keep in mind that within the ad lib period, a feeding gradient appeared, with an overall higher intake of the marine diets, which furthermore increased with NSP level (Intake increase of high NSP diets probably occurred to compensate for a lower energy content). The observed pattern, described below, probably would have occurred also for the plant diets if palatability was higher.

Nonetheless, the data show a lower fat digestibility in the marine diets during ad lib feeding. Furthermore, this decrease is more pronounced at higher NSP levels. This decrease in fat digestibility runs parallel to an increase in cholic acid output. In other words increasing feeding levels (probably beyond a certain threshold because the input of fat is higher, increasing the need for the emulsifying effect of bile salts) seem to drastically decrease fat digestibility and increase cholic acid output. The latter might be an explanation for the lower fat digestibility. The increase in cholic acid output was caused most likely by an increase in faecal output with higher NSP levels and not by direct binding of bile salts to NSP's since the concentrations of faecal bile salt did not change significantly with changing NSP levels.





Key words: *Oncorhychus mykiss*, bile salts, fat digestibility

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