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Plastic responses by wild brown trout (*Salmo trutta*) to plant-based diets

Stéphanie Céline Michl[a,b,c,*], Benjamin Weis[a], Jeffrey A. Hutchings[d,e], Carsten Schulz[a,b]

[a] Gesellschaft für Marine Aquakultur mbH (GMA) Büsum, Hafentörn 3, 25761 Büsum, Germany
[b] Institute of Animal Breeding and Husbandry, Department for Marine Aquaculture, Christian-Albrechts-Universität zu Kiel, Hermann-Rodewald-Str. 6, 24118 Kiel, Germany
[c] GEOMAR Helmholtz Centre for Ocean Research, Wischhofstr. 1-3, 24149 Kiel, Germany
[d] Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, Nova Scotia B3H 4R2, Canada
[e] Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, PO Box 1066 Blindern, NO-0316 Oslo, Norway

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ABSTRACT

Decreasing fishmeal availability and increasing prices promote the usage of plant-derived feedstuff as a substitution for fishmeal in commercial salmonid diets. However, little is known about the impact of plant-derived feedstuff on juvenile brown trout (*Salmo trutta*), a species that exhibits strong phenotypic plasticity with various genetic sub-structures and high overall genetic diversity. Thus, the production of brown trout for restocking purposes preferentially uses wild fish as broodstock to avoid loss of genetic variability. Because of nutritional programming, the strictly carnivorous feeding habit of wild brown trout broodfish could nevertheless have a negative impact on the digestive physiology of fry and fingerlings that are fed with commercial plant-protein containing trout diets. The present study, therefore, investigated whether the feeding of plant-based diets from first feeding onwards induced a permanent improvement in the utilisation of plant-derived protein sources in wild brown trout juveniles. Any plastic responses to the experimental diets resulting in a long-term physiological effect were hypothesised to be not only observed in growth performance, but also in altered pepsin and amylase activities. We demonstrated that (i) the feeding of wild brown trout fry with inclusion levels of up to 50% of dietary plant proteins is beneficial during the first weeks of life and (ii) continuous feeding of at least 50% plant-derived dietary protein resulted in the same rate of growth when compared to the growth resulting from fishmeal as the exclusive dietary protein source. Pepsin and amylase activities were only partly affected by diet-type and it can be concluded that intestinal pepsin and amylase activities in juvenile brown trout are primarily regulated by intrinsic mechanisms. In the present experiment, we were not able to induce a permanent nutritional programming effect of the first feeding diet; instead, a cross-over diet change applied 89 days post first feeding demonstrated that wild brown trout fry exhibit highly plastic responses to different feeding strategies during the first months of life.

1. Introduction

The production of brown trout (*Salmo trutta*) in aquaculture mainly focuses on juveniles for restocking purposes and increasing angling opportunities for recreational fisheries (FAO, 2012). The production of adult fish for human consumption is of minor interest: growth rates of brown trout are not comparable to rainbow trout (*Oncorhynchus mykiss*) or Atlantic salmon (*Salmo salar*) and, thus, production is economically less competitive (Gjedrem and Gunnes, 1978; Kizak et al., 2011). Furthermore, no family-based breeding program has been established for brown trout aquaculture, in contrast to Atlantic salmon or rainbow trout (Frank-Gopolos et al., 2015). Instead, wild fish are preferentially used as broodstock for restocking purposes to avoid loss of genetic variability (Quillet et al., 1992). Brown trout generally exhibit strong phenotypic plasticity with various genetic sub-structures and high overall genetic diversity (Ferguson, 1989). Juvenile brown trout are usually fed diets commercially manufactured for rainbow trout (FAO, 2012), which implies an increasing percentage of plant-derived feedstuff as a substitution for fishmeal (Hardy, 2013) because of decreasing fishmeal availability and increasing prices (Tacon and Metian, 2015). The effects of various dietary plant-based resources on growth and health of salmonids have been intensely studied in the last two decades (Baeverfjord and Krogdahl, 1996; Gomes et al., 1995; Shafaeipour et al., 2008; Slawski et al., 2012; Thiessen et al., 2003). It has also been demonstrated that the digestive physiology of fish can be heavily affected by plant-based diets, especially due to digestive enzyme

* Corresponding author at: Gesellschaft für Marine Aquakultur mbH (GMA) Büsum, Hafentörn 3, 25761 Büsum, Germany.
E-mail addresses: michl@gma-buesum.de, stephanie_michl@gmx.de (S.C. Michl).

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inhibitors or indigestible carbohydrates (Krogdahl et al., 2010). Nevertheless, most studies have focused on Atlantic salmon and rainbow trout, and little is known about the impact of plant-derived feedstuff on brown trout.

However, it is known from studies on gilthead sea bream (*Sparus aurata*) that the composition of diets for broodstock directly influences the utilisation efficiency of diets for juveniles because of *nutritional programming* (Izquierdo et al., 2015), a concept well known from studies on humans (Hanley et al., 2010) and becoming of increasing interest in aquaculture (Moghadam et al., 2015). *Nutritional programming* is dependent on an early-life stimulus that provokes a permanent or long-term physiological effect on later life stages (Hanley et al., 2010). Thus, the strictly carnivorous feeding habit of wild brown trout broodfish could impact the digestive physiology of fry and fingerlings that are fed with commercial trout diets containing about 50% plant-derived proteins. Moreover, *nutritional programming* is not only effective via reproduction: the early feeding with plant-based diets has been shown to significantly improve growth and feed intake of rainbow trout later in life when fed again with plant-based diets (Geurden et al., 2013).

The present study, therefore, aimed to induce *nutritional programming* in wild brown trout by feeding plant-based diets from first feeding onwards. It was hypothesised that the early feeding of plant-derived protein sources would act as a nutritional stimulus provoking improved growth performance when the same diets were applied later in life. Therefore, the potential of wild brown trout fry to exhibit plastic responses to different plant-based feeding strategies was explored and it was investigated whether the feeding of plant-based diets from first feeding onwards induced a permanent improvement in growth performance.

Digestive enzymes were additionally monitored from hatching onwards because of their substantial role in the nutrient digestion already of salmonid fry and for their known susceptibility to plant-based diets. Amylase, for example, is highly conserved between organisms and is a fundamental component of the enzymes present in the early development of piscivorous fish (Darias et al., 2006), although prey fish does not contain starch. Moreover, it has been demonstrated that amylase is associated with the gut microflora of fish because of carbohydrase-producing bacteria (Austin, 2006) and it has been shown that plant-based diets significantly alter the intestinal microbiome of first feeding rainbow trout (Ingerslev et al., 2014). Fish amylases are susceptible to anti-nutritive factors present in plant products. Proteinaceous amylase inhibitors are present in a variety of agricultural plants, such as wheat and beans (Marshall and Lauda, 1975; Silano et al., 1973), and the processing of plant meals for use in aquafeeds not necessarily eliminates proteinaceous enzyme inhibitors. For example, α-amylase inhibitory activities were even increased after dehulling of dry beans (Deshpande et al., 1982) and α-amylase inhibitors were still present in a protein concentrate from winged bean (Sathe et al., 1982). Thus, we hypothesised an impact of plant-based feed ingredients via anti-nutritive factors on amylase activities, even from highly purified protein sources.

It has also been shown that acidic digestive activity increases more rapidly in an early developing trout fry than alkaline digestive activity (Kitamikado and Tachino, 1960). This indicates that acidic digestion, including the key enzyme pepsin, is highly important in the first days after feeding. In contrast to marine fish larvae, salmonids possess a fully developed gastric digestion already at the onset of first feeding, which significantly improves protein assimilation efficiencies (Rust, 1995). Although the intestinal enzyme trypsin functions as the main enzyme involved in peptide digestion and is primarily affected by anti-nutritive factors, it has been shown in a gastro-intestinal model that the gastric digestion of proteins significantly influences subsequent bio-accessibility and bio-availability of nitrogen and phosphorous, in contrast to an exclusive alkaline digestion (Morales and Moyano, 2010). Moreover, another in vitro study demonstrated a significantly reduced nitrogen release from the protein hydrolysis of soybean meal compared to that of fishmeal by digestive enzyme extracts (acid and alkaline) of sea bream (Moyano and Savoie, 2001). Furthermore, in humans it has been observed that acid secretion and gastrin release were significantly reduced by isolated soy protein compared to beef protein (McArthur et al., 1988) and gastrin, a neuroendocrine hormone, stimulates the release of pepsinogen, the precursor enzyme of pepsin. This variance is attributed to the differences in structural characteristics of proteins derived from plants and those of animals as reviewed by Nehete et al. (2013): most proteins found in plants are globular, while those in animals are mostly fibrous. Posttranslational modifications additionally contribute to the various structural characteristics of plant storage proteins and, in contrast to trypsin, the cleavage efficiency of pepsin is directly affected by such structural differences. It is well-known from medical research that pepsin cleavage-efficiencies are strongly related to the size and the molecular structure of the food protein (Thomas et al., 2004) and thus can contribute to the allergenic potential of a protein source. Those observations make a continuous monitoring of pepsin activities in first feeding brown trout fry highly relevant when plant-based proteins are utilised in order to determine the potential impact of the dietary protein source on pepsin activity and thus on overall protein digestibility.

2. Material & methods

2.1. Experimental animals

The experiment was conducted at the “Gesellschaft für Marine Aquakultur mbH” (Büsum, Germany). Eyed brown trout eggs (*Salmo trutta*) were reproduced at the Fischbrutanstalt Altmühlendorf (Germany) from wild brown trout caught in Schleswig-Holstein. All animal handling procedures were approved by the animal welfare officer of the “Gesellschaft für Marine Aquakultur mbH” and the local authority of Schleswig-Holstein, according to the German animal welfare law (TierSchG).

2.2. Experimental diets

Three isoenergetic diets (diet X, diet Y and diet Z; see Table 1) were formulated and produced in cooperation with Skretting ARC (Stavanger, Norway). All diets were isonitrogenous in order to evaluate the effects of different protein sources on digestive enzyme activities without masking these effects by varying dietary protein levels. Fishmeal served as the exclusive protein source for diet X. In contrast, fishmeal contributed only 10% to the protein supply of diet Z. The remaining percentage was covered by various plant-protein sources. Diet Y is intermediate between diet X and diet Z with 50% plant-derived proteins and 50% fishmeal. The use of fishmeal as exclusive protein source in diet X furthermore required slightly higher starch contents compared to diets Y and Z in order to maintain isonitrogenous diets. All diets were extruded and crumbled in five different particle sizes from 0.5 to 3.0 mm to adjust feed particles according to the size of fry. The amino acid composition of each diet was formulated according to the NRC (2011) digestible amino acid requirements for small Atlantic salmon fry (0.2–20.0 g) and likewise was the composition of the vitamin and mineral premixtures in accordance with the NRC (2011) guidelines.

2.3. Chemical analysis of dietary nutrients and whole-fish body homogenates

The chemical nutrient analysis of all experimental diets and of whole-fish body homogenates was performed according to the European Commission Regulation (EC) No 152/2009 (European Union 2009). Dry matter content of whole-fish body homogenates was determined by freeze-drying whole fish for 72 h before homo-