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Evaluation of family growth response to fishmeal and gluten-based diets in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

A study was conducted to evaluate genotype × feed interactions in a commercial strain of rainbow trout (*Oncorhynchus mykiss*). Microsatellite DNA markers were used to determine the pedigree of the top 1% and bottom 1% of progeny in a large scale commercial growth trial of 24,000 rainbow trout from 20 full-sib families (20 dams × 10 sires in a nested mating design). The progeny were pooled at eyed stage and divided into 2 groups. Half of the fish from each family were fed a standard fishmeal-based diet and the other half was fed a plant protein (gluten)-based diet to determine the relative family rankings in each diet. The primary protein sources in the plant protein-based diet were corn gluten and wheat gluten meals. Krill was supplemented to this feed for the early life stages (starter, #1, #2, #3 crumbles), but was eliminated in the larger pellet sizes. Large genetic variation for growth was identified for both diets and the sire effect was found to be highly significant ($P<0.001$). The family rankings were similar for both diets, which suggest that the fish that grow faster on fishmeal diet are likely to grow faster on plant protein-based diets, and therefore current commercial strains that exhibit superior growth should retain their improved performance if raised on gluten-based diets. Multiplexing microsatellite markers would further improve the efficiency of parentage assignment protocols in large-scale rainbow trout selection programs.
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Keywords: *Oncorhynchus mykiss*; Selection; Plant diets; Breeding; Microsatellites; Parentage determination; Triploidy

1. Introduction

The aquaculture industry has received a large amount of criticism in recent years regarding the volumes of fishmeal and fish oils used in the manufacture of feeds, particularly for salmonid diets. Increasing concerns over potential negative environmental impacts, from both an effluent water quality standpoint

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and the use of wild-caught fish as feed ingredients (Goldberg et al., 2002), have prompted increased examination of alternate diet formulations for aquaculture (Gomes et al., 1995; Hardy, 1996; Sugiura et al., 1999; Carter and Hauler, 2000; Kissil et al., 2000). The anticipated changes in feed formulations have raised concerns regarding the ability of fish selected for rapid growth on traditional, fishmeal-based diets to effectively utilize these alternate diets (Blanc, 2002).

Genetic improvements in aquaculture species have been reported with increasing frequency in recent years (Gjedrem, 2000). Results from commercial rainbow trout breeding programs have shown gains of approximately 15% per generation in selections for body size (James Parsons, unpublished data). Many aquaculture selection programs utilize family-based mating design and benefit from the high fecundity of most aquatic species, external fertilization which enables simultaneous multiple matings and the use of semen storage and cryopreservation for delayed fertilization. Full- and half-sib families possess the appropriate genetic relationships for estimating breeding values and genetic correlations among traits of interest (Falconer and Mackay, 1996). However, difficulty in marking small aquatic species has often necessitated the rearing of early developmental stages in individual family tanks, resulting in shared tank effects by family members that were reared together (Winkelman and Peterson, 1994). Additionally, performance when the families are reared separately is not necessarily representative of the performance in mixed family tanks (Herbinger et al., 1999). The use of genetic markers for assigning parentage and for pedigree analysis in "common garden" aquaculture experiments has become fairly common (O'Reilly et al., 1998; Fishback et al., 1999, 2002; Herbinger et al., 1999; Hara and Sekino, 2003; Sekino et al., 2003; Rodzen et al., 2004; Vandeputte et al., 2004) and allows evaluation of genotype × environment effects without confounding common environment effects. However, the high cost of molecular biology techniques necessary to carry out these analyses has limited the use of "genetic tagging" by commercial breeders. A true cost–benefit understanding of the genetic improvement made by reducing this common environmental effect weighed against the cost of the molecular analysis is greatly needed.

The specific objectives of this paper were to evaluate family growth response to fishmeal and gluten-based diets in a widely used commercial strain of rainbow trout which were previously selected for improved growth when fed standard fishmeal-based diets and to assess the magnitude of genotype × diet interactions in rainbow trout.

2. Materials and methods

2.1. Fish stocks

A commercial strain from Troutlodge, Inc. (Sumner, WA, USA), which has been under intensive, family-based selection program for improved growth for several generations provided the base population for this study.

2.2. Mating design and early rearing

Twenty individual females were mated to ten males in the following manner: two females were randomly assigned to be fertilized by a single male. This process was repeated for all ten males until gametes from each of the 20 females were fertilized. Gametes were collected on a single day and held at 4 °C until fertilization. Ovarian fluid was drained from the eggs, and 150 ml of a buffered saline solution (5 mM Tris, 2 mM glycine, 0.5% NaCl) was added along with approximately 5 ml of the appropriate milt. The egg/sperm mixture was gently mixed, allowed to rest for 5 min and then excess milt solution was decanted off. Eggs were water hardened in a 50 mg/l povidone iodine solution for 20 min. After this initial water hardening period, ambient water flow (10 °C) was reintroduced to the incubation tray and development proceeded until the "eyed" stage.

At eyed stage, an estimate of egg size was made using water displacement, and 1200 eggs from each full-sib family were retained. The eggs from all 20 families (24,000 in all) were pooled, mixed well, and randomly split into two groups. Each of these groups was incubated separately in a vertical tray incubator through hatching until the point of initiation of feeding.

At the initiation of feeding each group was placed into a separate rearing container of adequate size and supplied with first use ambient spring water (12 °C). Feeding was accomplished as described below, and bi-weekly sampling of average weight continued throughout the grow-out period of 294 days (approximately 350 g bodyweight). Fish on each diet were reared in three raceways. A schematic illustration of the experimental design is shown in Fig. 1.

2.3. Diet formulation, and feeding regime

The formulation of each diet is shown in Table 1. The starter, #1, #2 and #3 crumble sizes of each of the