THE ROLE OF STARTER DIETS ON THE DEVELOPMENT OF SKELETAL ABNORMALITIES IN ZEBRAFISH

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Introduction

The occurrence of skeletal abnormalities is a significant problem for aquaculture industry (Boglione *et al*. 2013), as well as for laboratory fishes (e.g. zebrafish, Martins *et al*. 2018; Printzi *et al*. 2021). Skeletal abnormalities have severe consequences not only on the production cost but also on the welfare of the fish (Boglione *et al*. 2013). Most of the skeletal abnormalities develop during larval rearing and early juvenile period (Koumoundouros 2010). Main factors that trigger the development of skeletal abnormalities are the adverse abiotic conditions, genetic factors and nutrition (Boglione *et al*. 2013).

In this study, we examined the effect of different starter diets on the development of skeletal abnormalities and survival rate in zebrafish *Danio rerio*.

Material and Methods

Four commercial (A-D) and one experimental (CTRL) starter diets were tested. Of the commercial, two are formulated specifically for zebrafish rearing (A and D), one for freshwater ornamental fish (B) and one for marine fish larvae (C). Larvae were initially fed with *Artemia* nauplii (4-7 days post fertilization, dpf), then with *Artemia* nauplii and dry diets (8-11 dpf), and finally with dry diets only (12-20 dpf). Larvae were fed five times per day. Trials were performed in triplicate, in a closed recirculation system, at 28ºC water temperature.

At 18-20 dpf, a random sample of fifty specimen were taken from each experimental population anaesthetized, formalin fixed and double-stained for the examination of skeletal abnormalities (Walker and Kimmel 2007). For the survival rate, the total number of fish in each diet was counted at the end of the larval rearing. The significance of the differences in the abnormality rates and survival rate among the dietary regimes was tested by G-test (Sokal and Rohlf 1981).

All diets were analyzed with respect to their proximate composition (Table 1).

Results & Discussion

Our results showed that starter diets had a significant effect on the development of caudal scoliosis ($p<0.05$, 19.3-54.7%, Fig. 1B, B') and gill-cover abnormalities of either light ($p<0.05$, 11.3-36.7%, Fig. 1C, C') or severe ($p<0.05$, 1.3-11.3%, Fig. 1D, D') intensity. Similarly, the survival rate was significantly affected by the nutritional regimes applied ($p<0.05$, 63.1-90.3%, Fig. 1A) in the different diets.

Existing literature demonstrate that larval diet has significant effects on the development of zebrafish skeleton (Martins *et al*. 2018, Printzi *et al*. 2021). Our results showed that the use of specialized zebrafish diets (A, D, CTRL) may comparatively decrease the incidence of skeletal abnormalities and increase fish survival rate. More research is required to fine-tune the nutritional composition of zebrafish starter diets whilst taking into account critical rearing parameters on zebrafish skeletal development.

Table 1. Proximate composition of each starter diet.

Feed	DM (%)	Ash (%)	Proteins (%)	TL (%)	NL (%)	PL (%)
Artemia	94.8	6.5	61.7	19.2	9.2	6.6
Control	94.0	12.0	54.7	16.7	4.7	11.7
A	93.9	13.9	66.8	18.5	4.0	8.8
B	93.9	12.0	63.5	12.6	5.7	4.6
C	93.2	14.0	67.3	16.8	6.8	5.5
D	91.0	12.7	67.7	13.0	4.0	6.3

DM, dry matter; TL, total lipids; NL, neutral lipids; PL, phospholipids.

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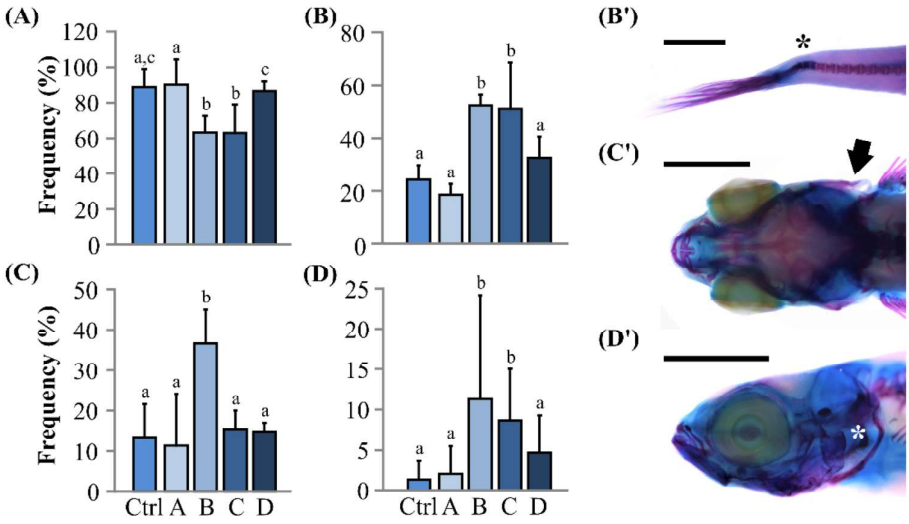

Fig. 1. Survival rate (A) and abnormalities frequency (B-D) in the five dietary regimes, at the end of the trials (18-20 dpf). (B-B') caudal scoliosis. (C-C') light gill-cover abnormalities. (D-D') severe gill-cover abnormalities. The absence of a common letter indicates statistically significant differences between diets (G-test, $p<0.05$). Error bars are equal to 1SD. Scale bars = 1.0 mm.

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