Full Paper

THE EFFECT OF DIFFERENT LEVELS OF DIETARY N-3 FATTY ACID ON THE REPRODUCTIVE PERFORMANCE OF ZEBRAFISH (*Brachydanio rerio*)

PENGARUH PERBEDAAN KANDUNGAN ASAM LEMAK N-3 DALAM PAKAN TERHADAP PENAMPILAN REPRODUKSI IKAN ZEBRA (*Brachydanio rerio*)

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Abstract

This experiment was conducted to determine the dietary n-3 fatty acid requirement for reproduction of broodstock zebrafish, *Brachydanio rerio*. Three isonitrogenous (39% crude protein) and isocaloric (3,260 kcal digestible energy/kg diet) practical diets, namely diets A, B, and C, with different levels of fatty acids were fed to zebrafish broodstock. The broodstock were cultivated in aquaria. Diet A contained low dosage of n-3 fatty acids (0.4%) and 2% n-6 fatty acids, while diets B and C contained 2% n-6 fatty acids, combined respectively with 1% and 1.5% n-3 fatty acids. Fish were fed *ad satiation* for 60 days using these diets. During feeding periode, gonade maturation stages were examined. The n-3 and n-6 fatty acids affected fecundity, fertilization rate, and hatching rate. On the other hand, fish fed on diets A, B, or C did not show any significance differences in the gonade somatic index and total Survival Rate (SR) of larvae produced. Fish fed on diet B produced the highest fecundity (616 eggs/g of fish), fertilization rate (94.6%), and hatching rate (93.8%). The total lipid content of eggs were significant, ranging from B (29.7%), C (23.7%), and A (16.1%). At a dosage of 2% n-6 fatty acids, zebrafish require 1% of dietary n-3 fatty acids in the diet for reproduction. Excess dosage of n-3 fatty acid in the diet adversely affected fecundity, fertilization rate, and hatching rate.

Key words: *Brachydanio rerio*, fatty acid, reproductive performance

Introduction

Recently, ornamental fish such as zebrafish (*Brachydanio rerio)* becomes a popular fish in Indonesia. For this reason, the seed demand has been increased. On the other hand, the existing hatcheries could not supply the seeds demand due to the low productivities. In order to improve the performance, good quality of broodstock is necessary. Nutrition is known to have a great influence upon gonadeal growth and fecundity in fish (Fernandez-Palacios *et al*. 1994; Izguerdo *et al*., 2001). The composition of broodstock diet is believed to have profound effects on the reproduction and egg quality of several fish (Watanabe *et al*., 1984; Fernandez-Palacios *et al*., 1994; Izguerdo *et al*., 2001). Moreover, for successful development the embryos of fish depend totally on the nutrients stored in the yolk.

Lipid and fatty acid composition of broodstock diet have been identified as major dietary factors that determine successful reproduction and survival of offspring (Izguerdo *et al*., 2001). Several investigations into the fatty acid metabolism of fish have been conducted. It has been shown that the fatty acid composition of a fish diet influences the fatty acid composition of the fish. The present results show the same relationship between fish diet, fatty acid composition of parental fish and fatty acid composition of their eggs. Fernandez-Palacios *et al*. (1994) concluded that zebrafish requires fatty acid components

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in the diet for egg production. Poly-unsaturated fatty acids can also regulate eicosanoid production, particularly prostaglandins, which are involved in several reproductive processes.

In freshwater fish, in general both the n-3 and the n-6 series of HUFA (Highly Unsaturated Fatty Acids) are nutritionally important. Freshwater fish commonly require n-6 fatty acid, or both n-6 and n-3 fatty acids, but different fish species require differ level group essential fatty acid (Watanabe *et al*., 1984). This experiment was conducted to determine the dietary n-3 fatty acid level in practical diet for reproduction of broodstock zebrafish *Brachydanio rerio*.

Materials and Methods

Experimental diets
Three practical diets were used in this experiment (Table 1).

The main protein source was fish meal and the alternative protein source were defatted soybean meal, pollard, and wheat flour. Pollard and wheat flour were used as the carbohydrate sources and binders. The lipid source was a mix of corn oil and coconut oil.

The broodstock rearing
Broodstock fish with body weigh 0,11-0,13 g were used in this experiment. The fish were reared at 26±1^{0}C under a 12 hours light and 12 hours dark (Maack & Segner, 2004). Ten females were placed in each aquaria (30x30x30 cm). Experiment was done in triplicates.

The fish were fed with the experimental diets four times daily (*ad satiation*). During feeding period, gonade maturation stages were examined. The fish begun to be mature after two months of the feeding trials. Spawning of the zebrafish took place when the light was switched on in the morning. Only healthy fish without diseases and abnormalities were used as parental fish for the production of fertilized eggs. The interval between spawning ranges from 2 to 5 days. The non-sticky eggs are 1.0-1.2 mm in diameter and have a transparent chorion. The eggs produced by the respected females were fertilized by natural fertilization and were incubated in aquaria. Sample of egg and larvae in one aquarium from each female were counted in order to get the hatching rate. This experiment was conducted for 2 months.

Table 1. Composition and proximate analysis of the experimental diets

Ingredients	Diets/ *n*-3 and *n*-6 fatty acids(%)		
	A (n-3:n-6 = 0.4:2)	B (n-3:n-6 = 1:2)	C (n-3:n-6 = 1.5:2)
Fish meal	31.0	31.0	31.0
Soybean meal	29.0	29.0	29.0
Pollard	19.5	19.5	19.5
Fish oil	0.0	1.0	2.3
Corn oil	3.3	3.3	3.2
Coconut oil	2.2	1.2	0.0
Vitamin mix*	2.0	2.0	2.0
Mineral mix*	3.0	3.0	3.0
Wheat flour	10.0	10.0	10.0
Protein	39.8	39.9	39.9
Lipid	10.30	10.15	10.19
Ash	8.52	8.39	8.51
Carbohydrate	41.39	41.47	41.40
DE (kkal/100g)*	326.17	325.86	325.69
C/P	8.20	8.15	8.16

* Takeuchi (1988)

Statistical analysis

This experiment used a completely randomized design.Gonade somato index (GSI), fecundity (F), fertilization rate (FR), hatching rate (HR) , and total number of larvae produced were subjected to one-way analysis of variance and Tuckey Test to determine significant differences among treatments (Steel &Torrie, 1980).

Chemical analysis

Proximate analysis was done on the experimental diets, eggs, and broodstock (whole body). Moisture, crude ash, crude protein (semimicro-Kjeldahl), crude lipid (Folch method), and carbohydrate were analysed as described by Takeuchi (1988). The fatty acid compositions of experimental diets were analyzed by gas liquid chromatography (GLC) using Fucad silica capillary column (GC-15A, Shimadzu Corp., Japan); at 50-205^{0}C with gradient of 4^{0}C/min as described by Takeuchi (1988).

Chemico-physical water parameters were recorded and regulated daily to ensure steady test conditions (Table 2).

Result and Discussion

Fecundity, hatching rate, and fertilization rate of diet B (n-3:n-6=1:2) was higher than that other diets; but the gonade somatic index and survival rate (3 days old larvae) of all treatments diets were the same (Table 3). The broodstock fed on diet A (n-3:n-6=0.4:2) produced the lowest fecundity, hatching rate, and fertilization rate (P<0,005). Table 4 shows the water and lipid content of egg. Table 5 shows proximate composition of the broodstock (whole body), and Table 6. shows fatty acid content of the experimentasl diets.

Table 2. Chemico-physical water parameters

Parameter	Content	Water Quality Measurement
Temperature (^{0}C)	26^{0}C±1	Termometer
pH	8.1±0.2	pH meter (HM-30V; Tao Electronics Ltd., Japan)
Oxygen (mg O_2/l)	6.5±1.5	DO meter (DO-20V; Tao Electronics Ltd., Japan)
Ammonia (ppm)	0.01±10.01	Test Kit (Sera ammonia test; Sera GmbH, Germany)

Table 3. The gonade somatic index (GSI), fecundity (F), hatching rate (HR), fertilization rate (FR), and survival rate (SR) produced by fish during the experimental period

Parameter	Diets/ *n*-3 and *n*-6 fatty acids(%)		
	A (n-3:n-6 = 0.4:2)	B (n-3:n-6 = 1:2)	C (n-3:n-6 = 1.5:2)
GSI (%)	23.64 ± 4.54 [a]	25.43 ± 1.96 [a]	24.79 ± 4.90 [a]
F (eggs/g of fish)	84.28 ± 4.60 [a]	616.53 ± 261.14 [c]	377.54 ± 57.14 [b]
FR (%)	52.78 ± 3.65 [a]	94.59 ± 3.12 [c]	75.71 ± 2.75 [b]
HR (%)	49.45 ± 2.31 [a]	93.97 ± 2.40 [c]	61.89 ± 3.11 [b]
SR_3 (%)	66.67±11.55 [a]	80.00 ± 20.00 [a]	73.33 ± 11.55 [a]

[1] Values in each row with the same superscript are not significantly different (P<0,005).

Table 4. The water and lipid content of egg (% dry weight)

Proximate composition	Diets/ n-3 and n-6 fatty acids(%)		
	A (n-3:n-6 = 0.4:2)	B (n-3:n-6 = 1:2)	C (n-3:n-6 = 1.5:2)
Water	56.54	66.81	61.21
Lipid	16.08	29.68	23.74

Table 5. Proximate composition of the broodstoct (% dry weigh)

Proximate composition	Diets/ n-3 and n-6 fatty acids(%)		
	A (n-3:n-6 = 0.4:2)	B (n-3:n-6 = 1:2)	C (n-3:n-6 = 1.5:2)
Protein	56.32	59.13	56.84
Lipid	16.70	9.76	14.03

Table 6. Fatty acid content of the experimental diets (%)

Ingredients	Diets/ n-3 and n-6 fatty acids(%)		
	A	B	C
n-3	0.41	1.13	1.52
n-6	2.08	2.11	2.07

Fecundity is the total number of eggs produced by each fish expressed in term of eggs/body weight. Reduced fecundity, reported in diet A (n-*3* : n-6 = 0.4:2) and C (n-*3 : n-6* = 1.5:2), could be caused either by the influence of a nutrient imbalance on the brain-pituitary-gonade endocrine system or by restriction in the availability of a biochemical component for egg formation (Izguerdo *et al*., 2001). Indeed, one of the major nutritional factors that have been found to significantly affect reproductive performance in fish is the dietary essential fatty acid content (Watanabe *et al*., 1984).

In general, the n-3 and n-6 fatty acid content of the broodstock diet would affect lipid content of egg produced. The lipid content of egg from diets B (n-3:n-6 = 1:2) 29.68% was higher than that of diets C (n-3:n-6 = 1.5:2) 23.74% and diets A (n-3:n-6 = 0.4:2)16.08% (Table 4). The n-3 and n-6 levels, and the n-3/n-6 ratio of the broodstock diets also affected the fecundity, hatching rate, and fertilization rate. During embryogenesis and larvae development, lipid was used as a source of energy; and egg of diet B (n-3:n-6 = 1:2) had the highest energy reserve. So, the n-3 and n-6 and then n-3/n-6 fatty acid ratio in diets (Table 6) would determine the success of embryogenesis, which could be seen in the value of hatching rate. The fatty acid composition will affect the membrane fluidity and permeability. Other function of the essential fatty acid has a role as a precursor of prostaglandin (Leray *et al*., 1985). Prostaglandins (PGs) are also recognized as important pheromones in some teleost fish. Some PGs produced by female fish, such as PGFs, have been shown to stimulate male sexual behaviour and synchronize male and female spawnings, thus directly affecting the success in fertilization (Sorensen *et al*., 1988). However, in this experiment the fecundity, hatching rate, and fertilization rate were low in excess or lack amount of n-3 fatty acid, as was shown by diet C (n-3:n-6 = 1.5:2)and diet A(n-3:n-6 = 0.4:2).

The above-mentioned lipid analyses of diets (Table 1) and eggs (Table 4) explain the lipid metabolism of zebrafish. This fish species is able to elongate and desaturate the fatty acid, the n-3 fatty acid as well as the n-6 fatty acid, applied with the diet. As Watanabe *et al* (1984) mentioned, dietary lipids affects the fatty acid composition of phospholipids to a greater degree than the fatty acid composition of triglycerides. In freshwater fish, dietary 18:2 n-6 is elongated and desaturated and converted to 20:4 n-6 and 22:5 n-6 fatty acid. In the phosphor-lipids fraction of fish the 18:3 n-3 fatty acid is elongated desaturated to 22:6 n-3, whereas in the triglycerides fraction, fatty acids are deposited unaltered, increasing the concentration of 18:2 n-6 and 18:3 n-3 (Izguerdo *et al*., 2001).

In summary, information on the nutrient requirements of broodstock fish is limited to a few species. Certain nutrients such as n-3 fatty acid have been shown to be particularly important in broodstock zebrafish nutrition. Their requirement during reproduction are higher than those of juveniles (Maack & Segner, 2004), but excess amount of n-3 fatty acid or an imbalance can be detrimental for reproduction.

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