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Research Article

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Effect of Dietary Protein Level on the Reproductive Performance of Female of Green Catfish (Hemibagrus nemurus Bagridae)

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Abstract

A study to determine the effect of increasing levels of dietary protein on green catfish (*Hemibagrus nemurus*), was carried out. Four isocaloric semi-purified diets containing 20%, 27%, 32%, and 37% dietary protein were used. Broodstock performance was evaluated based on growth parameters, reproductive parameters, and larvae production of female broodstock. Results showed that while the 20%, 27%, 32% and 37% protein produced the lowest specific growth rate (SGR) values, there was significant difference between 20%, 27%, 32% and 37% dietary treatments. The 20% dietary treatment also displayed longest time matured gonads and lowest of somatic ovi index and relative fecundity. Larvae production was highest from females fed with 32% and 37% protein, followed by the 27% protein while the diet containing 20% protein produced lowest number of larvae. Based on our results, we suggest that a minimum of 32% protein be included in the diet of female green catfish broodstock.

Keywords: *Hemibagrus nemurus*; Broodstock nutrition; Growth; Fecundity; Larvae

Introduction

Indonesia is one of the countries that are rich in biodiversity and recognized internationally as a 'hot megadiversity country'. Whithin 1,200 species of freshwater [1,2]. There are 260 species in inland waters include rivers, oxbow lake, lakes, reservoirs and floodplain in Riau Province [3]. In general, the species diversity has been decreased, Siak river was only found 36 species [4], Kampar Kiri river 86 species [5], Oxbow lake Serai 20 species [6], Kampar Kanan river 58 spesies [7], Kampar Kanan River 36 species [8], Koto Panjang reservoir 26 species [9] and small Giam Siak 37 species [10].

One species of fish that live in the inland waters of Riau Province which has important economic value is green catfish. This fish is highly favored by consumers because the thick meat, little prickly and the delicious taste, weight can reach sizes from 750 to 1000 g/individual so as to have a high market value i.e. 60,000 to 75,000 IRD/kg [11]. At the present time in Riau Province has been cultured with intensively by in cages farmers in river basin and ponds. But the rapidly development of cultured green catfish have not balanced by the high production because not supported by larvae production with good quality and quantity [12]. This is due, among others, the difficulty of getting broodstock mature gonads, Moreover, the results showed that green catfish fed with enriched vitamin E ranged from 150 to 450 mg/kg feed resulted in fecundity ranged from 23,750 to 30, 000 eggs/kg body weight [13], implantation with LHRH-a is still a little ranged from 20,815 to 32,000 eggs/ spawn [13], with 17-β-estradiol implantation ranged from 40,875 to 63,724 eggs/spawn [12].

Demand of green catfish juvenile age 50 days for cultured in Pekanbaru City and Kampar Regency is currently as 1,000,000 individuals every year, the majority of juvenile derived from wild fish catch and highly dependent on season and availability stocks. In order available at any time juveniles in quantity and quality for cultured to be supplied from hatcheries [12].

In the research, the author wants to analyze the effectiveness of the dietary protein levels on reproductive performance green catfish, because the nutrition of broodstock is one of the least studied areas because the biological mechanisms, such as gonadal maturation, are extremely complex processes [12,14-19]. The gonadal development and fecundity are affected by several nutrients, especially in continuous spawning fish with short vitellogenic periods as *Ictalurus punctatus* [18]. In general, the nutritional status of the female can influence gonadal development and limit the amount and the quality of the eggs [15,20,21]. The current study was designed to investigate the effects of dietary protein level on various reproductive aspects of green catfishl utilizing semi-purified diets.

Methodology

Broodstock and cages

Adult female and male green catfish (*H. nemurus*) were obtained from a commercial fish farm in Sungai Paku Village, Kampar Regency Riau Province and have been kept for >1.5 years in pond, avarage weight is 750 ± 100 g/individuals (Figure 1). The female of green catfish to be treated as individuals first twelve adapted for three months in the cages (2 × 1 × 1 m) with a stocking density of individual/cage. All cages are placed in pond (22 × 8 × 2 m) with an average water height of one meter. The pond water comes from Sungai Paku reservoirs to debit 0.2 m^3/second, pond water temperature ranged from 26°C to 28°C. Feeding was done twice daily and fish were fed a predetermined ration of 5% body weight day-1. During the adaptation of fish fed with commercial (pellets) with proximate composition are water content (% dry weight) 12.0%, crude protein 28.0%, lipid 5.5%, crude fiber 6.2% and crude ash 13.0%. Experiments conducted on green catfish female gonad maturity stage one with four groups and three replications. Each group given in the form of pellet feed with protein content of 20%, 27%, 32% and 37% respectively.

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The spawning green catfish conducted with GnRHa stimulation with dopamine antagonist at a dose of 0.5 ml/kg body weight and conducted artificial spawning. Hatching eggs each treatment and replication carried on fiber tubs (200 × 80 × 40 cm), height of water in the fiber tubs is 20 cm, water temperature during embryonic development ranged from 28 to 29°C, pH 6.8 to 7 and dissolved oxygen 5.6 to 6.8 mg/l. Egg samples from each treatment and replications preserved with Gilson solution. Gilson is preserved with a solution consisting 100 ml of 60% alcohol, 880 ml of distilled water, 15 ml of nitric acid, 18 ml of glacial acetic acid and 20 grams of mercury chloride. Furthermore, the diameter of the eggs was measured with a microscope Olympus CX 21 to 30 eggs of from each treatment and replications. The water temperature, dissolved oxygen (DO), and pH levels of the test chambers were regularly monitored.

Diets

Four semi-purified isocaloric diets with an increasing level of dietary protein (20%, 27%, 32%, and 37%) were formulated utilizing fish meal and soyben meal as protein sources (Table 1). Proximate analysis of diets was conducted according to Saraswanti Indo Genetech (2013) for verification of nutrient levels.

Checking the oocytes maturation

All fish were individually marked using floy-tags and weighed. Oocytes sampled in vivo were taken from females using the method described by Syandri [22], and were placed in Serra's solution (6:3:1, 70% ethanol, 40% formaldehyde and 99.5% acetic acid) for clarification of the cytoplasm. After 5 min, the position of oocytes nucleus was determined using a four-stage scale:

stage 1 germinal vesicle in central position

stage 2 early migration of germinal vesicle (less than half of radius)

Ingredient	Dietary protein levels (%)			

	20%	27%	32%	37 %
Fish meal (60% CP)[a]	21	30	36	40
soybean meal	10	12	15	18
fine bran	20	20	20	20
Dextrin	25	20	15	5
Shark liver oil	5	5	5	5
Corn oil	3	3	3	3
Vitamin premix[b]	2.5	1.5	1,5	1.5
Mineral premix[c]	2.5	1.5	1.5	1.5
CMC	1.5	1.5	1.5	1.5
Cellulose	8.5	5.5	5	4.5
Proksimat (dry weight)				
Crude protein (%)	20.2	26.05	31.8	37.1
Crude lipid (%)	9.5	9.8	10.1	10.3
Ash (%)	5.60	5.62	5.64	5.67
crude fiber (%)	4.5	4.6	4.70	4.75
Energy (k cal/g)	4.60	4.65	4.65	4.66

[a]Anugrah Sakti Fish Meal (crude protein: 60.0%, crude lipid: 13,0%, crude fiber: 1.5 %, crude ash 9.25%).

[b]Vitamin mix (mg/100 g feed): Thiamin-HCl 5.0; riboflavin 5.0; Ca-pantothenate 10.0; niacin 2.0; pyridoxin-HCl 4.0; biotin 0.6; folic acid 1.5; cyanocobalamin 0.01; inositol 200; p-aminobenzoic acid 5.0; menadion 4.0; vit A palmitat 15.0; cholecalciferol 1.9; α-tocopherol 20.0; cholin chloride 900.0 [c]Mineral mix (mg/100 g feed): KH_2PO_4 412; $CaCO_3$ 282; $Ca(H_2PO_4)$ 618; $FeCl_3.4H_2O$ 166; $ZnSO_4$ 9.99; $MnSO_4$ 6.3; $CuSO_4$ 2; $CoSO_4.7H_2O$) 0.05; KJ 0.15; Dekstrin 450; Selulosa 553.51.

Table 1: Composition and proximate analysis respectively of test diets.

stage 3 late migration of germinal vesicle (more than half of radius) stage 4 periphery germinal vesicle or germinal vesicle breakdown, GVBD (Table 1).

Parameters analyzed were

Specific growth rate (SGR %/day): [(lnWt-lnWi)/T)] × 100 where Wt=mean final weight, Wi=mean initial weight and T=total experimental days. Feed conversion ratio (FCR): total feed fed (g)/ total wet weight gain (g). Time mature gonadal calculated from the time the fish began dietary protein levels granted until the fish reaches a mature gonadal [days]. Ovi somatic index (OSI) was determined using the expression: OSI=BTO/BW × 100%; where BTO: ovulation egg weight and BW: Body Weight. Absolute fecundity (AF)=OVA × GW; where OVA: oocyte number per ovary gram and GW: Gonadal Weight. Relative fecundity was estimated using the formula: $RF=AF/BW$; where AF: Absolute Fecundity; BW: Body Weight.

Chemical analysis

The proximate composition of egg samples from each treatment was analysed using the following methods: moisture content by drying in an oven at 100°C for 24 h; protein using a Kjeltec Auto 1030 (Tecator Ltd.); and lipid by the Soxhlet method (AOAC., 1995). Egg samples for amino acid analyses were stored at -70°C before use. Total fry production: Total larvae harvested throughout experimental period (age 14 days).

Statistical analyses

Data were analyzed by one-way analysis of variance (ANOVA) and then tested using Dunnett-s test. All statistical analyses were performed using SPSS 17. (SPSS, Tokyo, Japan). In all tests, the level of significance used was 0.05.

Results and Discussion

Final weight, SGR and FCR

Growth of green catfish females dietary protein levels showed that while there was significantly ($p<0.05$) difference between final weight of diets 20%, 27%, 32% and 37% (Table 2 and Figure 1). Weight gain was lowest for the 20% protein diet, followed by 27%, 32% and 37% protein diets. Specific growth rate (SGR) was also significantly different between protein diet, SGR was lowest for the 20% protein diet, followed by the

Figure 1: Broodstock of *H.nemurus*.

27%, 32% and 37% dietary protein levels. Results from the FCR values also showed that the 32% and 37% dietary protein levels produced the highest efficiency in feed utilization (Table 2) (Figures 1-4).

The time sexual maturity and percentage of eggs weight

The mature gonadal of green catfish females dietary protein levels showed that while there was significantly ($p<0.05$) difference between the time sexual maturity (Figure 2 and Table 3). Average egg ovulation (SOI) was also significantly different between protein diet, SOI was lowest for the 20%, followed by the 27%, 32% and 37% dietary protein levels (Table 3).

Fecundity and eggs diameter

The mean absolute fecundity of green catfish females ranged from 40,033 to 64,650 eggs/spaw (Table 4). Absolute fecundity tended to increase with increasing level of diet protein. Increasing dietary protein levels also increase the relative fecundity and eggs diameter. The absolute fecundity, relative fecundity and eggs diameter were significantly ($p<0.05$) among the dietary protein levels (Table 4).

	Dietary protein levels			
	20%	27%	32%	37 %
Initial weight (g)	754.33 ± 14.50	755 ± 5	747 ± 11.53	754.66 ± 5.03

Final weight (g)	799.82 ± 13.50[a]	813.14 ± 6.89[b]	817.85 ± 13.56[c]	841.45 ± 6.82[d]
Weight gain (g)	44.82 ± 0.36[a]	58.14 ± 2.35[b]	70.85 ± 2.68[c]	86.79 ± 2.54[d]
SGR (%)	0.1 ± 0.002[a]	0.16 ± 0.008[b]	0.28 ± 0.02[c]	0.41 ± 0.03[d]
FCR	2.74 ± 0.16[a]	2.05 ± 0.14[b]	1.36 ± 0.18[c]	1.18 ± 0.07[d]

Table 2: Effect of different dietary protein levels on various growth parameters of female green catfish (mean ± SE).

Dietary protein levels	Average body weight (g)	Fecundity (number of eggs per spawn)	Relative fecundity (number of eggs per g gonadal weight)	Eggs diameter (mm)
20%	799.82 ± 13.50	40,033 ± 1,789[a]	49 ± 2[a]	1.07 ± 0.01[a]
27%	813.14 ± 6.89	46,896 ± 1,300[b]	57 ± 2[b]	1.11 ± 0.02[b]
32%	817.85 ± 13.56	58,106 ± 2,393c	70 ± 3[c]	1.16 ± 0.01[c]
37%	841.45 ± 6.82	64,650 ± 2,107[d]	77 ± 2[d]	1.21 ± 0.02[d]

Table 3: Effect dietary protein levels on time matured the gonads and percentage of eggs weight (mean ± SE).

Figure 2: Ovary stage IV of *H. nemurus*.

Figure 3: Larvae fourteen days.

Figure 4: Juvenile age 50 days.

Chemical composition of eggs and total larvae production

Percent protein, lipid and water content in the largest group of oocytes of stage 4 ovaries (post-vitellogenic oocytes; diameter ranged from 1.07 to 1.21 mm, Table 5). Protein and lipid (by dry weight) and water content ranged from 55.76 to 61.21% and 4.71 to 7.33%, respectively, and there was significantly differences ($P<0.05$) among the different dietary protein levels.

Mean total larvae production increased with an increasing dietary protein level. The highest larvae production was obtained with dietary protein levels of 32% (40,093 ± 2,100 individuals) and 37% (45,255 ± 2,950 individuals), followed by 27% (31,185 ± 3,105 individuals) and 20% (25,621 ± 2,659 individuals). The total larvae production were significantly ($p<0.05$) among the dietary protein levels (Figures 3 and 4 and Table 5).

Discussion

The green catfish (*H. nemurus*) females group dietary protein levels 20%, 27%, 32% and 37% can increased weight gain, specific growth rate and decrease feed conversion ratio. In contrast to redclaw crayfish (*Cherax quadricarinatus*) with dietary protein levels 22%, 27%, 32%, and 37% did not affect the final weight [17]. Numerous studies elsewhere have shown that an important contribution of dietary

protein toward broodstock performance is in the effect on the body size. Fish

Dietary protein levels	Average body weight (g)	Fecundity (number of eggs per spawn)	Relative fecundity (number of eggs per g gonadal weight)	Eggs diameter (mm)
20%	799.82 ± 13.50	40.033 ± 1.789^a	49 ± 2^a	1.07 ± 0.01^a
27%	813.14 ± 6.89	46.896 ± 1.300^b	57 ± 2^b	1.11 ± 0.02^b
32%	817.85 ± 13.56	58.106 ± 2.393c	70 ± 3^c	1.16 ± 0.01^c
37%	841.45 ± 6.82	64.650 ± 2.107^d	77 ± 2^d	1.21 ± 0.02^d

Values with the different superscript in each column are significantly different from each other ($p<0.05$).

Table 4: Effect of different dietary protein levels on fecundity and eggs diameter (mean ± SE).

Dietary protein levels	Egg protein (%) (dry weight)	Egg lipid (%) (dry weight)	Water content (%) (dry weight)	Average total larvae production
20%	55.76 ± 0.45^a	21.4 ± 0.55^a	7.33 ± 0.15^a	25,621 ± 2,659^a
27%	57.56 ± 0.20^a	18.33 ± 0.21^a	6.38 ± 0.10^a	31,185 ± 3,105^b
32%	58.20 ± 0.4^a	18.86 ± 025^a	7.27 ± 0.26^a	40,093 ± 2,100^c
37%	58.29 ± 0.36^a	17.33 ± 0.73^a	6.71 ± 0.11^a	45,255 ± 2,950^d

Values with the different superscript in each column are significantly different from each other ($p<0.05$)

Table 5: Effect dietary protein levels on percentage protein, lipid (by dry weight) and water content in post-vitellogenic oocytes (diameter 1.9-2.5 mm) of stage 4 ovaries and total larvae production (mean ± SE).

female swordtails (*Xiphophorus helleri* , Poeciliidae) feed with a protein content of 20%, 30%, 40%, 50% and 60% of dietary protein can also enhance final weight and weight gain [15]. In tilapia, female total weight at first maturation increases linearly with higher dietary protein levels [23]. The female tilapia attained puberty and oocyte maturation earlier when feed higher levels of dietary protein and concluded that this was due to the effect of diet on fish growth [20].

The green catfish (*H.nemurus*) females group dietary protein levels exposed can reaches the gonadal mature more rapidly, increased of the index ovi somatic, absolute fecundity, relative fecundity and egss diameter. With a 37% protein dietary level, capable of reaching a matured gonads of green catfish during 26 days and increase of fecundity as much as 64,650 eggs/individual. While the protein content of feed 20% of the time matured gonads achievement during 58 days and fecundity as much as 40,033 eggs/individual. The female dwarf gourami (*Colisa lalia*) broodstock showed higher total weight and higher number of oocytes undergoing vitellogenesis when feed higher dietary protein level [24]. The lower protein content obtained in both muscle and ovary of female swordtail fed with the 20% protein level probably indicates limited or insufficient protein for maintenance and oocyte development. Continuous feeding with a low dietary protein level also caused female tilapia to utilize body reserves during subsequent spawning seasons compromising muscle deposition and growth [20].

In general the exposed to 20% to 37% of dietary protein levels can increased ovi somatic index of green catfish ranged between 4.47 % to 8.24%. According to [13] that of green catfish exposed LHRHa to 400 µg /kg body weight to produced somatic ovi index 8.08%, and the exposed to 200 to 600 µg/kg body weight of 17ß-estradiol can increased ovi somatic index of green catfish ranged between 5.01% to 10.32% [12]. Green catfish were implanted 17 β- estradiol at a dose of 200 µg/ kg body weight reached matured gonads during 35 days and fecundity as much as 40,875 eggs/individual, whereas at a dose of 600 µg/kg body weight reached matured gonads during 26 days with fecundity as 52,500 eggs/individual [12]. The *Rhamdia quelen* females were given feed with protein levels of 28%, 34% and 40% had no effect on the main physiological and reproductive parameters. The dietary protein levels 28%, 34% and 40%, produced eggs are 127.00 ± 17.60; 140.00 ± 11.10; 143.00 ± 8.00 egg number ml-1 spawn respectively. In conclusion, the 28% crude protein dietary level was sufficient for maintenance of the broodstock and the reproductive indexes [19]. The fish reproduction is influenced by the quality of feed among others lipid, protein, fatty acids, vitamins E and C, and carotenoids as major nutrients influencing various reproduction processes such as frequency of spawning and the seed output, fecundity, fertilization, hatching and larval development [14,25], hormone levels 17-β-estradiol [26-28], hormone levels LHRH [29,30] and environmental factors [31]. In the present study, percent protein and lipid in the post-vitellogenic oocytes did not vary with the dietary protein level. Similar results were reported by [20] for tilapia (*Oreochromis niloticus*). But the levels of dietary protein significantly affect to larvae production until the age of 14 days. In summary, dietary protein levels had influence on weight gain, specific growth rate, feed convertion ratio, time matured gonadal, index ovi somatic, fecundity, eggs diameter and larvae production.

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