PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 14 (7): 412-416, 2015
ISSN 1680-5194

Effect of Energy, Lipid and Protein Content in Broodstock Diets on Spawning Fecundity and Eggs Quality of Giant Gourami (*Ospheronemus gouramy* Lac)

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Abstract: The purpose of this research was to know the effect of energy, lipid and protein in broodstock diets on spawning fecundity and eggs quality of giant gourami (*Ospheronemus gouramy* Lac). The study was consisted of three experiments, namely energy content experiment, lipid content experiment and protein content experiment. Latin Square Design with 4 treatments, 4 rows and 4 columns was used to analyze the data. The results of this research showed that: (1) content of energy in broodstock diets had highly significant effect ($p<0.01$) on spawning fecundity, eggs diameter, eggs fertility rate and eggs hatching rate, (2) content of lipid in broodstock diets had highly significant effect ($p<0.01$) on spawning fecundity, eggs fertility rate and eggs hatching rate, but it had not any effect ($p>0.05$) on eggs diameter, (3) content of protein in broodstock diets had highly significant effect ($p<0.01$) on spawning fecundity, eggs diameter and eggs hatching rate and it had significant effect ($p<0.05$) on eggs fertility rate. The best of energy, lipid and protein content in broodstock diet of giant gourami that was 3,300 kcal energy/kg diet, 5.0% lipid and 35% protein.

Key words: Energy, lipid, protein, broodstock, egg, giant gourami

INTRODUCTION

Giant gourami (*Ospheronemus gouramy* Lac) was a native fish in Indonesian freshwater that had spread to Southeast Asia (National Standardization Agency, 2000). It was a freshwater foodfish has high economic value and its meat taste was savory. It can be cultivated in ponds with limited land and water.

In cultivated of giant gourami, availability guarantee of quality seeds in sufficient quantity and continuously was an essential factor that determines the success of its business. Quantity and Quality of seeds was determined by the reproduction condition of broodstock. One of the factors that greatly effect broodstock reproduction was diet namely its nutrition content was such as energy, lipid and protein content. Nutrition deficiency in diet would lead to stunted growth and reproduction failure that ultimately decreased reproductive efficiency.

According Kjorsvik *et al*. (1990), one of main factors effected egg maturation and fecundity was the condition of the female broodstock. Conditions of female broodstock was strongly effected by the broodstock's own nutrition, therefore the right diet was important for reproduction performance of the female broodstock. Watanabe *et al*. (1984) expressed that the cultivation of broodstock with high-quality diet nutrition was important in obtaining high-quality fertile eggs. Then Knox *et al*. (1988) and Encina and Granado-Lorencio (1997) expressed also that the diet was good or quality will improve fish growth and provide the broodstock reached a larger size. Broodstock that larger would be produced eggs that are larger and a lot more and then the larger egg size will gave greater larval size.

The purpose of this research was to know the effect of energy content, lipid and protein in the broodstock diet on the quantity and quality of giant gourami eggs. From this research were expected to know the needs of energy, lipid and protein in female broodstock diet.

MATERIALS AND METHODS

This research used 48 female broodstocks and 48 male broodstock of giant gourami (*Ospheronemus gouramy* Lac) that 4 years old and weighing between 1,100 to 1,500 g. The materials were used in the manufacture of test diet that was fish meal, soybean cake, coconut cake, fine rice bran, wheat starch, tapioca starch and coconut oil. All diet materials were analyzed its nutritional content in Animal Science Faculty Laboratory of Andalas University Indonesia, namely protein, lipid, crude fiber and Gross Energy (Table 1). For completeness of vitamins and minerals were used Top Mix that was produced by Medion, Bandung, Indonesia.

This research consisted of three experiments, namely energy content experiment, lipid content experiment and protein content experiment.

Energy content experiment: In this experiment consisted of four energy treatments ie 2,300 kcal/kg diet (E_1), 2,800 kcal/kg diet (E_2), 3,300 kcal/kg diet (E_3) and 3,800 kcal/kg diet (E_4). In all four treatment diets lipid and protein were kept as similar as possible (5.0% lipid and

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25% protein) to keep this parameter from being a possible influence on reproduction. The feeding composition in the energy content experiment can saw in Table 2.

Lipid content experiment: This experiment consisted of four lipid treatments ie 2.5% (L_1), 5.0% (L_2), 7.5% (L_3) and 10.0% (L_4). In four treatment diets contained 3,300 kcal/kg energy content as this level yielded the best results in energy content experiment and protein content in this experiment was also kept a similar that is 25% protein. The composition and nutrition content in diets of the lipid content experiment can saw in Table 3.

Protein content experiment: This experiment consisted of four protein treatments ie 25% (P_1), 30% (P_2), 35% (P_3) and 40% (P_4). In four treatment diets contained 3,300 kcal/kg energy and 5.0% lipid content as this level yielded the best results in energy and lipid content experiment. The composition and nutrition content in diets of the protein content experiment can saw in Table 4.

Procedures and variables research: The each experiment consists of several stages: The first stage was the egg emptying stage of broodstocks that were conducted by the spawning twice. The second stage was the cultivation or maturation of the broodstock with diet of experiment according to treatment (content of energy, lipid and protein) until broodstock was mature gonads. The third stage was the broodstock spawning with comparison of male and female that was 1: 1, it was done naturally. The four stage was egg incubation of the result spawning of third stage in aquarium with eggs density was 75 eggs/L of water.

Variable that was measured in each experiment, namely:

1: Fecundity Spawning was the number of eggs were produced by the female broodstock that expressed as the number of eggs/kg of body weight
2: Egg diameter was measured by vernier caliper (accuracy 0.05 millimeters) under a microscope at a magnification 20 times
3: Fertility rate of eggs was the percentage of eggs that can be fertilized by sperm, it was calculated by the following formula:

$$\text{Fertility rate}\,(\%) = \frac{\text{No. of fertilized eggs}}{\text{No. of total eggs}} \times 100\%$$

4: Hatching rate of eggs was the percentage of eggs that can hatched after incubation, it was calculated by the following formula:

$$\text{Hatching rate}\,(\%) = \frac{\text{No. of hatched eggs}}{\text{No. of fertilized eggs}} \times 100\%$$

Statistical analysis: Each experiment used a Latin Square Design with 4 treatments, 4 rows (discharge time gonads) and 4 columns (initial weight of the broodstock). Data were analyzed by analysis of variance and Duncans multiple range test.

RESULTS

Energy content experiment: The effect of energy content in broodstock diets on spawning fecundity, eggs diameter, eggs fertility and eggs hatching rate is showed in Table 5.

Statistical analysis showed that feeding with different energy content had highly significant effect ($p<0.01$) on the spawning fecundity of broodstock. The highest spawning fecundity that was 3,115±165.2715 eggs/kg fish in the 3,800 kcal/kg energy treatment, whereas the lowest spawning fecundity that was 1,630±67.1536 eggs/kg fish in the 2,300 kcal/kg energy treatment. And then, there was not significant difference ($p>0.05$) between spawning fecundity of 3,300 kcal/kg energy treatment and spawning fecundity of 3,800 kcal/kg energy treatment, but both treatments had a much higher spawning fecundity than those fed the 2,300 kcal/kg energy content diet ($p<0.01$) and 2,800 kcal/kg energy content diet ($p<0.05$) (Table 5). Energy content also had highly significant effect on eggs diameter, where the largest eggs diameter that was 2.53±0.0295 mm in the 3,800 kcal/kg energy treatment and the smallest eggs diameter that was 2.40±0.0334 mm in the 3,300 kcal/kg energy treatment. And there was not significant difference ($p>0.05$) between eggs diameter of 3,300 kcal/kg energy treatment and eggs diameter of 3,800 kcal/kg energy treatment (Table 5). This experiment result also indicated that feeding with different energy content had highly significant effect ($p<0.01$) on the eggs fertility and hatching rate of broodstock. The highest eggs fertility and hatching rate that was 83.54±2.2615% and 84.90±3.4911%, respectively in the 3,800 kcal/kg energy treatment, whereas the lowest eggs fertility and hatching rate that was 70.52±3.0612% and 68.52±3.7819%, respectively in the 2,300 kcal/kg energy treatment. There were no significantly different ($p>0.05$) in eggs fertility and hatching rate between broodstocks were fed to contain energy of the 2,800 kcal/kg diet with broodstocks were fed to contain energy of the 3,300 kcal/kg diet and broodstocks were fed to contain energy of the 3,800 kcal/kg diet (Table 5).

Lipid content experiment: The effect of the lipid content in broodstock diets on spawning fecundity, eggs diameter, eggs fertility and eggs hatchability of this experiment is showed in Table 6.

Statistical analysis found that feeding with different lipid content was highly significant effect ($p<0.01$) on the spawning fecundity of broodstock. The highest spawning fecundity that was 2,986±201.5083 eggs/kg

Table 1: Nutrition content of experiment feed materials

Material	Gross energy (Kcal)	Lipid (%)	Protein (%)	Crude fiber (%)
Fish meal-1	1,598.51	2.47	26.83	6.43
Fish meal-2	2,795.34	2.66	45.93	5.89
Soybean cake	3,968.76	1.30	49.66	3.02
Coconut cake	4,256.98	14.23	24.45	15.46
Fine rice bran	4,106.37	12.69	10.13	12.63
Wheat starch	3,830.40	1.32	18.83	-
Tapioca starch	3,351.14	0.85	3.53	-
Coconut oil	8,792.52	100.00	-	-

Analysis result of Animal Science Faculty Laboratory Andalas University

Table 2: Feeding composition on the each treatment in the energy content experiment

	Treatment diets			
Material	E_1	E_2	E_3	E_4
Diet composition				
Fish meal-1 (%)	69.50	47.30	24.50	3.25
Soybean cake (%)	9.00	14.50	22.00	24.50
Coconut cake (%)	4.00	8.05	9.00	15.75
Fine rice bran (%)	-	5.00	14.00	15.25
Wheat starch (%)	3.00	12.50	19.00	34.25
Tapioca starch (%)	10.00	9.00	8.75	5.00
Coconut oil (%)	2.50	1.65	0.75	-
Vitamins (%)	1.00	1.00	1.00	1.00
Minerals (%)	1.00	1.00	1.00	1.00
Nutrition content				
Protein (%)	25.01	25.04	25.00	25.06
Lipid (%)	5.03	5.03	5.02	5.07
Crude fiber (%)	5.36	5.36	5.40	5.31
Gross energy (kcal/kg)	2,308.27	2,805.05	3,309.73	3,800.46

Table 3: Feeding composition on the each treatment in the lipid content experiment

	Treatment diets			
Material	L_1	L_2	L_3	L_4
Diet composition				
Fish meal-1 (%)	20.50	27.50	32.50	37.25
Soybean cake (%)	21.25	18.50	19.25	21.00
Coconut cake (%)	1.50	-	-	-
Fine rice bran (%)	7.25	7.90	6.00	3.75
Wheat starch (%)	40.50	40.50	32.00	21.00
Tapioca starch (%)	7.00	1.00	3.00	7.00
Coconut oil (%)	-	2.60	5.25	8.00
Vitamins (%)	1.00	1.00	1.00	1.00
Minerals (%)	1.00	1.00	1.00	1.00
Nutrition content				
Gross energy (kcal/kg)	3,318.51	3,311.64	3,317.75	3,325.24
Protein (%)	25.03	25.03	25.02	25.00
Crude fiber (%)	3.11	3.32	3.43	3.50
Lipid (%)	2.51	5.07	7.51	10.01

fish in the 5.0% lipid treatment, whereas the lowest spawning fecundity that was 2,625±153.2671 eggs/kg fish in the 5.0% lipid treatment. There were not significant difference ($p>0.05$) between spawning fecundity of the 5.0% lipid treatment with the 7.5% lipid treatment and the 10.0% lipid treatment (Table 6). The result of lipid content experiment indicated that there were not effect of lipid content in broodstock diets on fish eggs diameter ($p>0.05$) (Table 6). The result of this experiment indicated that feeding with different lipid content was highly significant effect ($p<0.01$) on the eggs fertility and hatching rate of broodstock. The highest

Table 4: Feeding composition on the each treatment in the protein content experiment

	Treatment diets			
Material	P_1	P_2	P_3	P_4
Diet composition				
Fish meal-2 (%)	40.00	47.10	50.00	50.90
Soybean cake (%)	9.50	7.00	20.00	32.70
Coconut cake (%)	-	-	-	-
Fine rice bran (%)	5.50	6.00	2.00	-
Wheat starch (%)	-	20.00	7.10	-
Tapioca starch (%)	40.25	15.40	16.00	11.30
Coconut oil (%)	2.75	2.50	2.90	3.10
Vitamins (%)	1.00	1.00	1.00	1.00
Minerals (%)	1.00	1.00	1.00	1.00
Nutrition content				
Gross energy (kcal/kg)	3,311.65	3,342.77	3,336.67	3,371.86
Lipid (%)	4.98	5.00	4.97	4.98
Crude fiber (%)	3.34	3.74	3.80	3.99
Protein (%)	25.07	30.03	35.00	40.02

eggs fertility and hatching rate that was 82.60±2.5582% and 86.27±3.0721%, respectively in the 5.0% lipid treatment, whereas the lowest eggs fertility and hatching rate that was 58.52±3.9731% and 65.33±2.2896%, respectively in the 10.0% lipid treatment. There were no significantly different ($p>0.05$) in eggs fertility and hatching rate between broodstocks were fed to contain 2.5% lipid with broodstocks were fed to contain 5.0% lipid (Table 6).

Protein content experiment: The result of this experiment is showed in Table 7.

Statistical analysis indicated that feeding with different protein content was highly significant effect ($p<0.01$) on the spawning fecundity of broodstock. The highest spawning fecundity that was 3,827±155.7689 eggs/kg fish in the 40% protein treatment, whereas the lowest spawning fecundity that was 2,868±114.9288 eggs/kg fish in the 25% protein treatment. There were not significant difference ($p>0.05$) between spawning fecundity of the 35% protein treatment with the 40% protein treatment (Table 7). This experiment also found that protein content in broodstock diets had highly significant effect ($p<0.01$) on fish eggs diameter. The largest eggs diameter that was 2.63±0.0334 mm in the 40% protein treatment and the smallest eggs diameter that was 2.48±0.0349 mm in the 25% protein treatment. The eggs diameter between the 30% protein treatment with the 35% protein treatment and the 40% protein experiment was not significant different (Table 7). For eggs fertility and hatching rate, the content of protein in broodstock diets effect significantly ($p<0.05$) on eggs fertility rate, but it effect highly significant ($p<0.01$) on eggs hatching rate. The highest eggs fertility and hatching rate that was 91.05±3.2731% and 92.33±2.6870%, respectively in the 40% protein treatment, whereas the lowest eggs fertility and hatching rate that was 83.37±3.6695% and 83.85±2.4995%, respectively in the 25% protein treatment. Then there

Table 5: Effect of energy on spawning fecundity, eggs diameter, fertility rate and hatching rate in *Ospheronemus gouramy* Lac

	Treatment (Gross energy content)			
Parameter	E_1 (2,300 kcal/kg)	E_2 (2,800 kcal/kg)	E_3 (3,300 kcal/kg)	E.4 (3,800 kcal/kg)
Spawning fecundity (eggs/kg body weight)	1,630±67.1536^{A}	2,546±165.4867^{Ba}	3,015±255.8881^{Bb}	3,115±165.2715^{Bb}
Egg diameter (mm)	2.40±0.0334^{Aa}	2.46±0.0217^{ABb}	2.51±0.0421^{Bc}	2.53±0.0295^{Bc}
Fertility rate (%)	70.52±3.0612^{Aa}	78.39±2.6772^{ABb}	82.26±2.2320^{Bb}	83.54±2.2615^{Bb}
Hatching rate (%)	68.52±3.7819^{Aa}	79.38±3.1114^{ABb}	85.97±2.5874^{Bb}	84.90±3.4911^{Bb}

Mean (±SE) in the same row with different capital letters superscripts are highly significant different (p<0.01), whereas mean (±SE) in the same row with different small letters superscripts are significantly different (p<0.05)

Table 6: Effect of lipid on spawning fecundity, eggs diameter, fertility rate and hatching rate in *Ospheronemus gouramy* Lac

	Treatment (Lipid content)			
Parameters	L_1 (2.5%)	L_2 (5.0%)	L_3 (7.5%)	L_4 (10.0%)
Spawning fecundity (eggs/kg body weight)	2,625±153.2671^{A}	2,986±201.5083^{B}	2,931±105.3658^{B}	2,854±113.7629^{B}
Egg diameter (mm)	2.45±0.0238^{a}	2.50±0.0259^{a}	2.53±0.0286^{a}	2.52±0.0259^{a}
Fertility rate (%)	80.26±3.1389^{Aa}	82.60±2.5582^{Aa}	73.13±3.7506^{Ab}	58.52±3.9731^{Bc}
Hatching rate (%)	83.03±3.6801^{Aab}	86.27±3.0721^{Aa}	77.60±4.1929^{Ab}	65.33±2.2896^{Bc}

Mean (±SE) in the same row with different capital letters superscripts are highly significant different (p<0.01), whereas mean (±SE) in the same row with different small letters superscripts are significantly different (p<0.05)

Table 7: Effect of protein on spawning fecundity, egg diameter, fertility rate and hatching rate in *Ospheronemus gouramy* Lac

	Treatment (Protein content)			
Parameters	P_1 (25%)	P_2 (30%)	P_3 (35%)	P_4 (40%)
Spawning fecundity (eggs/kg body weight)	2,868±114.9288^{Aa}	3,336±116.4291^{ABb}	3,729±183.5710^{Bc}	3,827±155.7689^{Bc}
Egg diameter (mm)	2.48±0.0349^{Aa}	2.57±0.0396^{ABb}	2.62±0.0492^{Bb}	2.63±0.0334^{Bb}
Fertility rate (%)	83.37±3.6695^{a}	87.16±2.9415^{ab}	89.85±3.2139^{b}	91.05±3.2731^{b}
Hatching rate (%)	83.85±2.4995^{A}	89.25±2.1692^{B}	90.60±1.7365^{B}	92.33±2.6870^{B}

Mean (±SE) in the same row with different capital letters superscripts are highly significant different (p<0.01), whereas mean (±SE) in the same row with different small letters superscripts are significantly different (p<0.05)

were no significantly different (p>0.05) in eggs fertility and hatching rate between broodstocks were fed to contain 30% protein with broodstocks were fed to contain 35 and 40% protein (Table 7).

DISCUSSION

Energy content experiment: Increasing of the energy content in the broodstock diet could increase significantly spawning fecundity (eggs production), diameter, fertility rate and hatching rate of eggs. De Silva and Anderson (1995) stated that energy content in diet is needed for everything that a fish does from basic metabolic functions to swimming and reproduction. The more energy provided in a diet over and above basic needs may then be allocated to growth and reproduction. When limited energy is available, fewer eggs may be produced, but the more energy that is made available, the more eggs will be able to yield until maximum reproduction is achieved. Watanabe *et al.* (1984) in Memis and Gun (2004) also stated that high energy level had a great effect on broodstock spawning. Then Brown (2009) stated that for ensure good egg production it is necessary to feed a diet that provides the nutrients and energy for growth and health of the fish as well as for the developing oocytes. The research result of Badger (2004) on Rainbowfish (*Melanotaenia splendida splendida*) showed that raising the energy content from 11 mj/kg (2,627.30 kcal/kg) to 17 mj/kg (4,060.38 kcal/kg) in broodstock diets could increase eggs production from 55±8.4 eggs to 255±15.0 eggs, fertility rate from 79.4±4.5% to 99.9±0.9% and hatching rate from 76.4±5.4% to 98.7±0.6%.

Lipid content experiment: Lipid content in broodstock diets had highly significant effect on spawning fecundity (eggs production), fertility rate and hatching rate of eggs, but it had not effect on eggs diameter. The increasing of spawning fecundity, fertility and hatching rate of eggs occurred from the lipid content level of 2.5 to 5.0% and then they decreased at the lipid content level of 7.5 and 10.0%. Although the lipid content levels was high, but they could not increase fecundity spawning, fertility and hatching rate of eggs. This condition was caused by lipid content in broodstock diets that was already her requirement to reproduce. Fernandez-Palacios *et al.* (1997) stated that lipid is important to fish reproduction, but if too much lipid in a diet can be detrimental to egg quality. Too little lipid or poor quality can lead to a decrease in ovary size (MacFarlane *et al.*, 1993) and lower egg survival to hatching (Navas *et al.*, 1998). Besides that it was also caused by the composition of the fatty acids in the broodstock diet treatments that was not suitable with the requirement of the broodstock in the reproduction process. Navas *et al.* (2001) stated that quality of the eggs was not effected by the increase of amount of lipid in the diets, but it was effected by the fatty acid composition in diets.

Protein content experiment: The 35 and 40% protein diet treatments had similar reproductive success. The 25% protein diet treatment gave the lowest values of reproductive success in terms of spawning fecundity, diameter, fertility rate and hatching rate of eggs. The 35% protein diet was deemed the more ideal diet in this

experiment. Dahlgren (1980) showed that high feed protein levels (33%) increased reproduction success in *Poecilia reticulate*. Then experiment of Badger (2004) also showed that 35% protein diet gave increasing of reproduction in *Melanotaenia splendid*. Abidin *et al.* (2006) evaluated three different protein levels (30, 35 and 40%) of diet on the bagrid catfish (*Mytus nemurus*) and it were found higher fecundity in treatment with 35 and 40% protein level, concluding that 35% protein level was adequate to support egg quality parameters. Some research results suggested that a minimum 30% protein should be included in the diet of female broodstock for optimum reproductive performance in Swordtail, *Xiphophorus helleri* (Chong *et al*., 2004), in grass carp, *Ctnenopharyngodon idella* (Khan *et al*., 2004). El-Sayed *et al*. (2003) showed increased dietary protein levels increased the total number of eggs produced per spawn in tilapia, *O. niloticus*. Increasing protein levels in diet also improved fertilization and hatchability of fish eggs in *O. niloticus* (Gunasekera *et al*., 1996).

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