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A Lipid-walled microcapsule diet as co-feed for early feeding the *Osphronemus gourami* (Lacepede) larvae

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ABSTRACT. The effects of microcapsule diet as co-feeding for early weaning phase of *gourami* larvae where evaluated in terms of ingestion rate of microcapsule, growth, and survival rate. The availability of tubifex worms as natural food is uncertain and can introduce pathogens to fish culture from outside environment. Therefore, the attempt to replace or substitute this natural food continues in order to reduce the level of dependence. This research used Complete Randomized Designed (CRD) with four treatments based on a different protocol feeding management. *Gourami* larvae should be started with the co-feeding on day 12 until day 17 and they are more receptive to microcapsule diets after day 17. The ingestion rate of microcapsule which consumed by the larvae during the period of rearing was 48.33, 68.33, and 88.33%. This study indicated that larvae started co-feeding day 22 showed highest of ingestion rate of the microcapsule, survival rate, specific growth rate and weight gain.

Keywords: *gourami*; feeding management; microcapsule; fish larvae; growth; survival rate.

Dieta micro encapsulada com lipídeos (*lipid-walled)* como co-alimentação na alimentação precoce da larva de *Osphronemus gourami* Lacepede

RESUMO. Os efeitos da dieta de microcápsula como uma alimentação na etapa precoce de transição das dietas do *gourami* larvae foram avaliados sobre a taxa de ingestão da microcápsula, crescimento e taxa de sobrevivência. A disponibilidade das minhocas *tubifex* como um alimento natural é incerta e pode introduzir patógenos externos para na alimentação dos peixes. Assim sendo, a tentativa de trocar ou substituir esse alimento natural continua com a finalidade de reduzir os níveis de dependência. Esta pesquisa usou o método de Complete Randomized Designed (CRD) com quatro tratamentos baseados em protocolo diferentes manejos de alimentação. *Gourami* larvae foram introduzidas na co-aliementação entre os dias 12 até 17, quando as larvas são mais receptivas à dieta de microcápsula. A taxa de ingestão da microcápsula consumida pela larva durante o período de criação foi de 48.33, 68.33, e 88.33%. Este estudo indicou que a larva que começou a ser co-alimentada no dia 22 mostrou uma maior taxa de ingestão de microcápsulas, taxa de sobrevivência, crescimento especifico e ganho de peso.

Palavras-chave: *gourami*; gestão da alimentação; microcápsula; larvas de peixe; crescimento; taxa de sobrevivência.

Introduction

Gourami (*Osphronemus gourami*) is one of the local freshwater fish species with high potential for propagation by fish farmers, especially in the area around Banyumas District, Central Java, Indonesia. *Gourami* has high economic value, easily cultivated, and favored by consumers in Indonesia. Many studies were conducted for increasing and maintain fish production in Indonesia (Prayogo, Wijayanti, Murwantoko, & Astuti, 2012; Prayogo, Siregar, & Sukardi, 2016; Prayogo, Wijayanti, Sulistyo, & Sukardi, 2016). In traditional way aquaculture in Indonesia, low survival *Gourami* larvae during the early stages feeding often related to nutrient deficiencies thus their growth is poor. At the early stage development of *Gourami* larvae are fed live food, such as tubifex worm and daphnia. The content of this natural feed showed highly variable in quality and nutritional composition. Tubifex and daphnia usually enriched with whole-egg chickenemulsions to improve the nutritional quality of the live feed (Sukardi, Hana, Isdy, & Edy, 2011).

Various studies had been conducted to improve the quality and quantity of artificial feed for larvae (Cahu & Infante, 2001; Koven, Kolkovski, Hadas, Gamsiz, & Tandler, 2001). However, generally some artificial feed that was developed has a weakness,

which is less durable in storage and easy to crumble that can contaminate the culture media (Kvåle et al., 2006; Rosenlund, Stoss, & Talbot, 1997; Yúfera, Kolkovski, Fernández-Dıaz, & Dabrowski, 2002). Degradation study of micro diet are still needed to understand the larval digestion process, producing bases for the future development of artificial diets that can replace live prey (Fernández-Diaz & Yufera, 1997; Langdon, 2003; Tesser & Portella, 2003). A study dealing with degradation of microcapsule diet has been done in fish larvae (Yúfera et al., 2002), and in bivalve species, *Crassostrea virginica* (Chu, Webb, Hepworth, & Casey, 1987).

Microcapsules capable of being an alternative to replace the use of natural food for fish larvae (Jones, Holland, &Jabborie, 1984; Langdon, 2003), include Gilthead Seabream, *Sparus aurata* L. (Gamsız & Alpbaz, 2015), Larval Red Drum (*Sciaenops ocellatus*) (Lazo, Dinis, Holt, Faulk, & Arnold, 2000), Senegal sole (Yúfera et al., 2003), cod (Kvåle et al., 2006), striped bass (Chu & Ozkizilcik, 1999) and Halibut (*Hippoglossus hippoglossus* L.) (Murray et al., 2010). Other studies, microcapsule for larvae of mollusks the Pacific Oyster, *Crassostrea gigas* (Langdon, 1989) and crustaceans, Penaeid shrimp (Jones, Kurmaly, & Arshard, 1987). Microbound diet was also used as an alternative for larvae of *Litopenaeus vannamei* (D'Abramo, Perez, Sangha, & Puello-Cruz, 2006). Chitosan-walled microcapsule diet to know the morphology of capsule, leaching of total nutrients and free amino acids (FAA) and diet acceptance of *Macrobrachium rosenbergii* (Anas, Philip, & Singh, 2008). Gelatin-walled microcapsule diet was evaluated to know the retention of vitamin C, lipid encapsulation and nitrogen retention efficiency for *Penaeus japonicus* Bate (Xie et al., 2010). Microencapsulated diet using different wall materials was evaluated its special effect on the activity of digestive enzyme and growth performance of *Penaeus japonicus* Bate (Xie et al., 2011), and *Nibea albiflora* larvae (Xie et al., 2015).

This study aimed to determine the rate of feed ingestion of microcapsules on *Gourami* larvae (*O. gourami*) with a different starting time of co-feeding, growth and survival rate of the larvae. In this study, we improve the quality and quantity of artificial feed for *gourami* larvae with a Lipid-walled microcapsule diet.

Material and methods

Larval rearing and experimental design

Larval rearing was carried out in the closed water systems. A total of 1,600 larvae, 9 days post-hatchingwere used in this experiments. The fish larvae were obtained from hatchery of the wet laboratory of Faculty of Biology, Jenderal Soedirman University. Water temperature, dissolved oxygen, pH and alkalinity were monitored daily. Water temperature was maintained between 26–29°C. During the larval culture period, oxygen, pH and alkalinity were maintained at > 85%, 7.8 and 75–85 mg L^{-1}, respectively. Water exchange rate increased gradually with the age of the larvae.The quality of water including temperature, dissolved oxygen, pH and alkalinity were monitored and checked with U-50 multiparameter water quality checker.

Complete randomized designed (CRD) was used with four treatments and replications. Sixteen glass larval tanks (50x30x30cm≈ 45 L) were used and each tank contained of one hundred larvae which newly hatch of *O. gourami*.

Feeding management

O. gourami had egg-yolk until days eight or nine, after this period they start to eat. In this experiment, the larvae of which 9 days post-hatching used as starting point to given feed (Figure 1). The control group larvae, without co-feeding process, they werefed only *Tubifex* worm until the end of the experiment. Larvae with co-feeding which were fed mix *Tubifex* and microcapsules started on day 12 as the weaning trial 1, were fed started on day seventeenth as the weaning trial 2 and started on day 22 as the weaning trial 3. All larvae which were fed early with microcapsule until day 32. The remaining materials from microencapsulated diet and fecal were taken with siphoning the water half hour after larvae were fed.

Preparation of tubifex extract

Tubifex worms homogenized and then were centrifuged at 1200 rpm for 15 minutes. Supernatant collected from centrifugation used as the components of inclusion and matrix.

Production of microcapsule feed

Lipid-walled microcapsules with aroma and flavor Tubifex wereutilized to deliver nutrients to larvae of Giant *Gourami* (*O. gourami*). Microcapsules particles produced using a modification method of the suspension cross-linking technique, as described by Chu et al. (1987) and Arshady (1989)was applied in this experiment. Tubifex aroma used as an attractant as well as the inclusion (core materials). Lipid-walled capsules were prepared by mixing one part of the matrix (wall) with one part of inclusion. The composition of microencapsulated feed for experimental feed (Table 1) as follows:

Table 1. Composition of microencapsulated diet for feeding experiment.

Diet	Diet components	% composition by weight
1	Matrix: (72.6% w^{-1})	
	fish oil	68.97
	extract *Tubifex* worm	3.63
	Inclusion: (27.4 % w^{-1})	
	egg-yolk	5.75
	extract*Tubifex*	21.63
	vitamins B1, B2, B12	0.014

Microcapsule size.

After production, the diameters of 100 capsules were measured microscopically in order to determine mean capsule size.

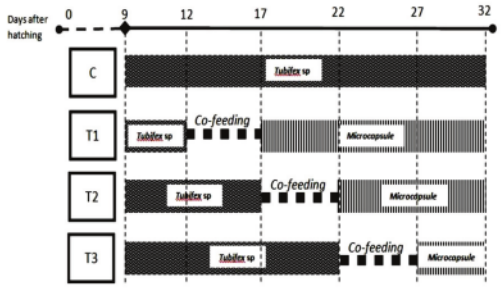

Figure 1. Early weaning protocol of a feeding management of *O. gourami* larvae during the experiment (C= control group, without co-feeding precess, they were fed only Tubifex until the end of experiments, T1= larvae with co-feeding which were fed mix tubifex and microcapsules started on day 20 as the weaning trial, T2= were fed started on day 17 as the weaning trial, and T3= were fed started on day 12 as the weaning trial).

The leaching test of the microcapsule feed

The rate of loss of the microcapsules was measured by modified analytical methods and a spectrophotometer, using distilled water as the medium (Chu et al., 1987; Önal & Langdon, 2000). Phenol red, a water-soluble dye, was used to assess rupture of the lipid microcapsule wall and the rate of loss of water-soluble nutrients from inside the capsule. The phenol red was added to aqueous solution used to prepared the lipid wall microcapsule. We thought that phenol red and water-soluble nutrients, such as vitamins, were leached from the capsule at comparable rates. Different concentrations of phenol red were used as standards. Preparation of microencapsulated diet for leaching experiments (Table 2).

In the leaching test, 100-mg microcapsules were inserted into a container containing 50 mL distilled water and soaked for 0, 1, 2, or 4h, using three repetitions for each time point. The absorbance of the filtrate was measured at 400 nm using a spectrophotometer (Spectronic 20D). The optical density of the filtrate measured at 400 nm using a spectrophotometer (Spectronic 20D).

Table 2. Composition of microencapsulated diet for leaching experimente.

Diet	Diet components	% composition by weight
2	Matrix: (72.6% w^{-1})	
	fish oil	68.97
	extract *Tubifex* worm	3.63
	Inclusion: (27.4 % w^{-1})	
	egg-yolk	5.75
	extract *Tubifex*	21.63
	vitamins B1, B2, B12	0.014
	phenol red	0.014

Ingestion rate

Ingestion rate of trial 1 was taken when the larvae aged 12, 17 and 22 days, respectively, whereas trial 2 was taken when the larvae aged 17, 22 and 27 days, respectively. And ingestion rate of trial 3 was taken when the larvae aged 22, 27 and 32 days, respectively. A total of 20 larvae from each trial was taken at 30 min after feeding. Larvae were anesthetized using 70% alcohol, then dissected under a microscope. A total of 20 larvae from each trial was taken at 30 min after feeding. Larvae were anesthetized using 70% alcohol, then were dissected under a microscope. The feed existence which consumed seen in the digestive tract by using a stereo microscope with light. The rate of digestion of larvae in each trial aquarium was calculated by dividing the larvae which had microcapsules in the digestive tract divided by the number of larvae were sampled. Percentage of ingestion (Chu & Ozkizilcik, 1999) was calculated using the formula as follows:

% Ingestion = [(Σ fish larvae consume microcapsules)/(Σsample of larvae)] X 100

Growth parameter

Growth parameter was calculated as follows: WG(g) = Wt + Wi, where WG = weight gain (g), Wt is final weight (g) and Wi is initial weight (g).
LI = length increment (cm), LI = Lt + Li, where Lt is final length (cm), Li is initial length (cm).

$$SGR\ \left(\%_d\right) = \frac{100[\ln \mathrm{Wt} - \mathrm{Wi}]}{T}$$

where Wt is final weight (g), Wi is the weight of fish at time 0, T is a culture period in days of the experiment.

Statistical analysis

For each of the four experimental trials, a one-way ANOVA using the general linear model procedure applied for each of the response variables to determine whether significant differences existed

among trials. Survival and percentage of larval variables subjected to the arcsine square root transformation before analysis. If significant differences found, then Turkey's procedure used for mean separation to determine which trials were significantly different from one another. All significant levels were set at $p < 0.05$, statistical analysis performed using SPSS for Windows (v. 11).

Results

Characteristic of the microcapsule diet

The microcapsules diameter produced ranged from 9.8 to 120 μm (means 58.9 μm) (Figure 2). The capsules in the digestive track of *O. gourami* larvae (Figure 3) ranged between 9.8 – 120 μm, whilst the length of larvae ranged between 1.10 – 1.99 cm (12 – 32 day of the hatch). Most of the larvae picked out 49-98 μm in diameter.

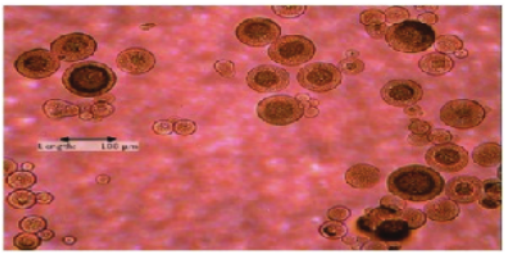

Figure 2. A Microphotograph showing the lipid-walled microcapsule (light microscope Boeco 10 x 10).

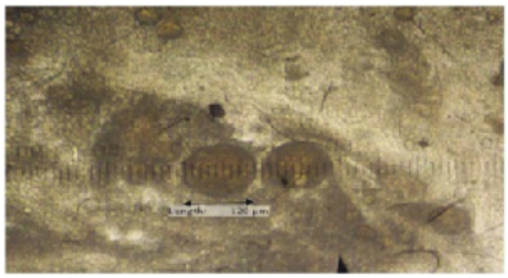

Figure 3. A Microphotograph showing the lipid-walled microcapsule in the gut larvae of *O. gourami* (light microscope Boeco 10 x 40).

Leaching of microcapsule feed

The following graph describes the relationship between absorbance at 400 nm and different concentrations of phenol red as standard curves (simple regression $r^2 = 0,9285$).

The optical density measurements of the filtrate from lipid-walled microcapsules containing phenol red were shown in Table 3. The rate of leaching was ranging from 0-4 hours still are relatively low, at 1.75% - 5:44%. There were no evident of leaching at 0 h, and then began increased after 1h ($p < 0.05$). However, the leaching after 2h was not significant different compare to 1h soaking ($p > 0.05$). The leaching rate of the microcapsules 4h soaking was significantly different compare to 2, 1 and 0h soaking, respectively. This indicates that the duration of soaking in the water increased followed by the rate of leaching significantly.

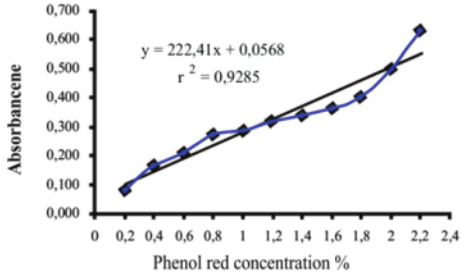

Figure 4. Standard curve for the absorbance of different concentrations of phenol red.

Ingestion rate of the control treatment did not compare with other weaning trials. However, based on observations, the rate of ingestion of *gourami* larvae that consume Tubifex 100% ingested. The rate of microcapsule ingested by the larvae of *Gourami* increased by the difference in weaning treatment (Figure 5).

Table 3. Optical density of lipid-wall microcapsules.

Soaking time (hours)	Optical Density	Filtrate Concentrations (%)	Decay Rate (%)
0	0,213 ± 0,044^a	0,702 x 10^{-3}	1.75
1	0,349 ± 0,029^b	1,313 x 10^{-3}	3.28
2	0,403 ± 0,018^b	1,557 x 10^{-3}	3.28
4	0,541 ± 0,002^c	2,177 x 10^{-3}	5.44

Filtrate was phenol red in the inclusion of microcapsules. Different superscript letters in the same columns denote mean ± SD, n = 5, means not sharing a common superscript are statistically different at $p < 0.05$.

O. gourami larvae with co-feeding started on day12 (weaning trial 1) showed lowest ingestion rate (48.3%) compare to larvae with co-feeding started on day 17 (weaning trial 2) was 68.3% and co-feeding started on day 22 (weaning trial 3) was 88.3%, respectively. Larvae with co-feeding started on day 17 was not different compared to larvae with co-feeding started on day 22, whilst larvae with co-feeding started on day 12 was significantly different compare to larvae started on day 22 ($p < 0.05$). This means trial of weaning can provide significant effect on the ingestion rate of microcapsule consumed by the larvae during period of rearing.

The proximate composition of microcapsule diet presented in Table 4. The dried microcapsules

contained 51.5 ± 0.07% crude protein, 22.4 ± 0.18% crude lipid and 15.6 ± 0.06% nitrogen-free extract. The average value of crude fiber and ash content was 2.01 ± 0.02% and 5.4 ± 0.11% of the dried microcapsules respectively.

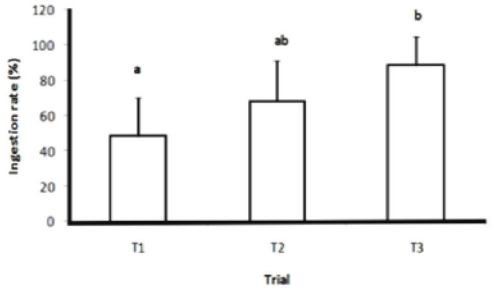

Figure 5. Ingestion rate of lipid-walled microcapsules of *O. gourami* larvae. Bars represented by different superscript letters indicate significantly different values ($p < 0.05$).

Table 4. Proximate composition of microcapsule diet dry matter basis, mean values with standard deviation, as determined by laboratory analysis (n=2).

	% dry matter
Crude protein	20.00 ± 0.43
Crude lipid	7.03 ± 0.29
Crude fibre	0.50 ± 0.03
Ash	2.50±0.55
Moisture	3.00 ± 0.15
NFE	70.00±0.45

Growth and survival of larvae

The growth of *O. gourami* for period of 12 weeks of weaning trials showed in Table 5. There was no significant difference in the length increment of *Osphronemus* larvae which were fed *Tubifex* worm or microcapsules diets. However, in the weight gain, larvae fed *Tubifex* then started co-feeding on day 12 (weaning trial – 1) and larvae started co-feeding on day 17 (weaning trial – 2) were significantly lower ($p < 0.05$) compare to larvae started co-feeding on day 22 and control group (Figure 6). Whilst larvae started co-feeding on day 22 was not different in the weight gain ($p > 0.05$) compare to the control group. In this experiment showed that microcapsule diet was more appropriate as a larval diet for this species started co-feeding on day 22 until day 27 or given microcapsule only after day 27.

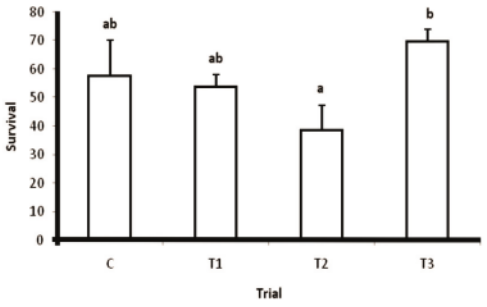

Figure 6. Survival rates of *O. gourami* larvae reared during 12 weeks of culture. Bars represented by different superscript letters indicate significantly different values ($p < 0.05$).

The specific growth rate (SGR) of *Gourami* larvae which started co-feeding on day 22 had greatest of mean of biomass SGR namely 8.213 ± 0.377 day^{-1} compare to larvae started co-feeding on day 12 and 17 had biomass SGR namely 6.187 ± 1.321 and 5.898 ± 0.729 day^{-1}, respectively.

Discussion

The experimental diet in this study was formulated taking into account the bibliography on larval fish nutritional requirements and diet formulations (Koven et al., 2001). Lipid-walled microcapsules appeared consumed after an initial period of adaptation.

This occurred when the diet introduced on day 17 and 22 post-egg-yolk. For the early introduction before day 17 post-egg-yolk, the larvae did not consume the microcapsules well (only 48.3% fill gut).

The rate of ingestion of *Osphronemus* larvae indicated that larvae more receptive to the microcapsules after day of 22. This occurred because of the larvae getting older, increased in weight and body size, phase which more complex has been done, as well as the mouth can be opened wider.An increase in the size of fish larvae leads to an increase in the mouth opening so that the larvae find it easier to feed (Black & Pickering, 1998; Fernández-Díaz, Pascual, &Yúfera, 1994).

Table 5. The growth of *O. gourami* during 12 weeks of experiment.

Parameter	Control	Trial 1	Trial 2	Trial 3
Initial weight (g)	0,015 ± 0,002^a	0,015 ± 0,001^a	0,015 ± 0,001^a	0,015 ± 0,001^a
Final weight (g)	0,107 ± 0,012^a	0,060 ± 0,007^b	0,068 ± 0,016^b	0,104 ± 0,008^a
Weight gain (g)	0,092 ± 0,012^a	0,046 ± 0,007^b	0,053 ± 0,016^b	0,090 ± 0,008^a
Initial total length (cm)	1,140 ± 0,036^a	1,100 ± 0,026^a	1,140 ± 0,016^a	1,090 ± 0,035^a
Final total length (cm)	2,000 ± 0,095^a	1,750 ± 0,089^a	1,8 ± 0,211^a	1,985 ± 0,138^a
Length increment (cm)	0,860 ± 0,099^a	0,640 ± 0,082^a	0,660 ± 0,203^a	0,895 ± 0,157^a
SGR (%)	8,123 ± 0,608^a	5,898 ± 0,729^b	6,187 ± 1,321^b	8,213 ± 0,377^a

Values in the same columns different superscript letters denote mean ± SD, n=3. Means not sharing a common superscript are statistically different at $p < 0.05$.

Halibut larvae have slow behavior in the ingestion of feed particles, causing a loss of nutrients from feed during ingestion. In contrast, Cod larvae can ingest the feed particles quickly so that the nutrient content, especially the protein is intact (Kvåle et al., 2006). Striped bass (*Moronesaxatilis*) larvae that are 14 days post hatching can do 100% ingestion Artemia and microcapsule diet (Chu &Ozkizilcik, 1999).

Other studies showed that increase in body weight in the early growth will accelerate the rate of feed ingestion of *Solea senegalensis* (Parra & Yúfera, 2001). Co-feeding will increase ingestion rates of microdiet when given together with Artemia for *Sparus aurata* larvae (Kolkovski, Koven, & Tandler, 1997), *Lates Calcarifer* Bloch (Curnow, King, Partridge, & Kolkovski, 2006). The larvae of sea bream with a total length of 4.5 mm would choose food particles under 50–150 μm and longer size larvae (>4.5 – <6 mm) would choose 151–250 μm, whilst larvae with a total length of 6 mm would ingested particles greater than 250 μm (Fernández-Diaz & Yufera, 1997). The accuracy of microparticle size for larvae was an important factor due to the development of opening mouth. Feed particles should be small enough for small fish and a diameter of less than 50 μm would be easy to ingested and digested (Cahu & Infante, 2001).The exact size of the diet given to fish larvae will facilitate the digestion and reduce food waste (Walford, Lim, & Lam, 1991). Several factors affected to the ingestion rate of microcapsules, such as size of larval (small or large), the mouth opening of larvae, a visual and chemical stimulus, texture (hard or soft), flavor and color of microcapsules (Fernández-Diaz & Yufera, 1997; Kolkovski, Tandler, & Izquierdo, 1997; Yúfera, Pascual, & Fernández-Dıaz, 1999). The physiological response to the larvae can be stimulated by natural feed so that the ingestion rate of microcapsules is increased when given together with natural feed (Koven et al., 2001).

Specific growth rate (SGR) of larvae which started co-feeding on days 12 and 17 got lower SGR when were compared with control and which weaned on the day of the 22 (the weaning experiment, 3). These showed that microcapsule diets more appropriate as a larval diet of *Osphronemus* after day 22. The study histochemistry of structure and function of digestive tract organ of *Osphronemus* completely built and especially stomach was perfect to accommodate food on day 22 after hatch (Mokoginta, 1995). Therefore, digestion and assimilation processes would be better when microcapsule is given starting end day 22. Microcapsule which started given to larvae on day 22 had higher survival rate, this phenomenon indicated that microcapsule suited given to the larvae at this time. Other studies showed that barramundi (*Latescalcarifer*) larvae weaned at age 4 to 28 days post hatching by co-feeding for 5 days resulted in a better specific growth rate (95 mg% per day), compared with co-feeding for 3 days (23 mg% per day),whilst the development of larval digestion was estimated at the age of 13 days post hatch(Curnow et al.,2006). Early weaning through co-feeding was also performed on bullseye puffer(*Sphoeroidesannulatus*), at 31 – 35 days post hatch (García-Ortega, Abdo, & Hernández, 2003).

Conclusion

The early weaning for *O. gourami* larvae should be started with the co-feeding (a combination of Tubifex and microcapsules) on day 22 until day 27 and given microcapsule only after days 27. Selected materials should be easy to digest and have high nutritional value for microcapsules production. These materials important due to the digestive tract of *Gourami* larvae not yet complete and fully functional during two or three weeks of its life.

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