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-Review-

Feeding and nutrients requirement of Sultan fish, *Leptobarbus hoevenii*: A review

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Abstract: Sultan fish (*Leptobarbus hoevenii*) is a commercially important freshwater fish with high potential for aquaculture production in the Southeast Asian countries, including Malaysia. Many studies have been focused on its nutrition and trophic biology but the feeding practices in the farming have not yet been reviewed. This paper reviews on nutritional management of *L. hoevenii* broodstock, larvae and juveniles in culture systems. In general, there are feeding guidelines developed for the *L. hoevenii* farming but they are not fully supported with the scientific studies. Knowledge on the larval developmental biology is lacking to fully understand the feeding ecology of this fish. In addition, there is a paucity of data on requirements of various major nutrients in *L. hoevenii* at different life stages. Further studies on these topics are essentially required to improve the feeding practices in the farming of *L. hoevenii.*

Keywords: Hoven's carp, Jelawat, aquaculture, nutrition, growth

Introduction

Sultan fish or the Jelawat (*Leptobarbus hoevenii*), is a native cyprinid that can be found in the rivers and lakes in Malaysia, Cambodia, Indonesia, Laos, Thailand, and Vietnam (Mohsin and Ambak, 1983; Roberts, 1989; Rainboth, 1993; Vidthayanon *et al*., 1997; Kottelat, 2001). It is one of the high value freshwater fish species cultured in these countries (Mohsin and Ambak, 1983; Kottelat *et al*., 1993) due to successful captive breeding in 1980s (Meenakarn, 1986; Saidin *et al*., 1988; Liao *et al*., 2000). In Malaysia, *L. hoevenii* has been recommended for human consumption due to its high contents of protein, some minerals (calcium, phosphorus, and iron) and vitamin B (Tee *et al*., 1989). In fact, the aquaculture production of this fish in recent years from 2015 – 2018 also has been steadily increased from 923 to 1771.28 tonnes (Fisheries Department of Malaysia, 2015 – 2018) to fulfill the market demand. Feeding plays a critical role in the farming of aquatic animals. There is no dearth of data on feeding *L. hoevenii* but this information has not been managed in a way to enable synthesis of useful data of potential use in aquaculture. This paper describes, evaluates, and provides an overview on the feeding practices in the farming of *L. hoevenii* and highlights the relevance of knowledge management of nutritional data for use in aquaculture.

Maturation Diets for Broodstocks

Dietary nutrients play an essential role in fish maturation and reproductive performance. Knowledge on the nutritional requirement of the brood fish is, therefore, critically needed for the maturation diets development (Luquet and Watanabe, 1986; Izquierdo *et al*., 2001).

In the feeding practices for the *L. hoevenii* broodstock, the fish are usually fed with the self-formulated feeds containing at least 30% of crude protein. According to Meenakarn (1986), feeding the *L. hoevenii* brood fish with the formulated diet containing 30% crude protein at 3% of the fish body weight (BW) daily can be practiced to prepare the fish for induced spawning. Truong *et al.* (2003) also reported their success in conditioning the *L. hoevenii* brood fish for induced spawning by feeding them 35% crude protein containing diet at the rate of 2% - 4% fish BW daily. Table 1 shows the ingredients and composition of the maturation diets for *L. hoevenii*

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Tab. 1: Ingredients and their composition in the maturation diets for *L. hoevenii* brood fish.

Parameters	Meenakarn (1986)	Truong *et al*. (2003)
Fish meal	35%	√
Soybean meal	25%	N/I
Rice bran	20%	√
Copra cake	10%	N/I
Wheat flour	6%	N/I
Vitamin premix	1%	√
Mineral	3%	
Blood powder	N/I	√
Fish oil	N/I	√
Crude protein level	30%	35%
Supplementary diets	A	B

√ = Included in the diet formulation but the amount was not provided
N/I = Not included
A: Vegetables at 10% of body weight; twice a week
B: Guava and plum at 2% of body weight

available from Meenakarn (1986) and Truong *et al*. (2003). These feeding practices are in agreement with the findings by Pathmasothy (1983), stating that dietary crude protein levels can significantly affect the gonad-somatic index (GSI) and fecundity of the *L. hoevenii*. Pathmasothy (1983) reported that the *L. hoevenii* brood fish fed diets containing 32% or 40% of crude protein level were found to attain significantly higher (P<0.05) GSI and fecundity than those fed diet with crude protein level of 24%; however, the optimum dietary protein requirement was not determined. Further study should be conducted to determine the optimum dietary protein requirement in the maturation diets for *L. hoevenii*, as well as the effect of different dietary amino acids on the *L. hoevenii* maturation and reproductive performance.

In addition to the high protein diets, Meenakarn (1986) supplemented vegetables to the *L. hoevenii* brood fish at 10% of their BW a week, while Truong *et al.* (2003) provided them guava and plums at 2% of their BW (without mentioning the frequency in the report). The intention of such a feeding protocol was to imitate the natural diets of *L. hoevenii*, since this fish is omnivorous (Roberts, 1993). However, the nutritional roles of plants in the diet of *L. hoevenii* is unknown. It is assumed that the *L. hoevenii* may utilize energy derived from the plants diet to maintain their active metabolism, while the protein obtained from the formulated feeds was used mainly for growth and maturation. Indeed, Montgomery and Targett (1992) reported that the omnivorous pinfish, *Lagodon rhomboids*, could partially assimilate the eelgrass diet for energy to maintain its active metabolism, while prioritizing the consumed protein from the grass shrimp diet for its optimum growth. This hypothesis should be elucidated in the future as the metabolic strategies of fish can be species specific (Chew and Ip, 2014; Lefevre *et al*., 2014), even among different cyprinids (Liew *et al*., 2012).

Other than protein, the dietary lipid source is another critical factor to be considered in the formulation of maturation diets for fish. Different lipid sources provide different essential fatty acids (EFA), and this is the key factor that influences the fish maturation and its reproductive performance (Izquierdo *et al*., 2001). The EFAs that are required in the maturation diets for the brood fish can be estimated from the fish eggs because the brood fish will incorporate the EFA they obtained from the diets into their gonads and eventually into the developing eggs (Izquierdo *et al*., 2001; Suloma and Ogata, 2012). In the case of *L. hoevenii*, the EFAs composition that are required to be in their maturation diets is still unknown. Nevertheless, the cyprinids (common carp, *Cyprinus carpio* and grass carp, *Ctenopharyngodon idella*) were reported to require both linoleic (C18:2n-6) and linolenic (C18:3n-3) acids through their diets (Takeuchi, 1996) because they lack enzymes to de-saturate the *de novo* synthesized oleic acid (C18:1n-9) to these EFAs (Henderson, 1996). In fact, both of these essential fatty acids were also detected in the eggs from the wild-caught *L. hoevenii* (Aryani *et al*., 2009). These outcomes suggest that the lipid sources with high linoleic and linolenic acids content should be used in the maturation diets for *L. hoevenii* brood fish. Further study is needed to validate this hypothesis.

Vitamins and minerals are also important nutrients for gonad maturation in fishes (Volkoff and London, 2018). However, there is still no information on the dietary vitamin and mineral requirements for the maturation diets of *L. hoevenii*. Further studies on this topic are necessary to improve the formulation of maturation diets for this fish.

Feeding Regimes in Larval and Juvenile Rearing

Optimum first feeding timing

In most of the fish species, the newly hatched fish larvae rely on yolk for the early development. When the yolk is almost exhausted, fish larvae make use of their functional sensory organs, especially the pigmented eyes for active feeding. At the same time, the larval mouth opens, and the digestive system develops. These ontogenetic developments prepare the fish larvae for the first exogenous feeding (Kawamura and Mukai, 1984; Kawamura and Ishida, 1985; Kawamura and Washiyama, 1989; Kawamura *et al*., 2003; Mukai *et al*., 2008; Mukai *et al*., 2010; Yahaya *et al*., 2011; Lim and Mukai, 2014; Silva *et al*., 2016). If the yolk is completely exhausted and the first feeding is delayed, the larvae will eventually reach succumb to starvation (Blaxter and Hempel, 1963; Yin and Blaxter, 1987). Determination of the optimum first feeding timing is, therefore, critically important to ensure high larval survival (Kross and Bromage, 1990; Kailasam et al., 2007; Ching *et al*., 2011; Chai *et al*., 2014; de Lima *et al*., 2017).

According to Truong *et al.* (2003), the arrangement for first feeding of *L. hoevenii* can be made one day after the larvae hatch out from the eggs (water temperature was not reported). However, Meenakarn (1986) suggested that the first feeding shall be made on day 3 as the *L. hoevenii* larvae only will be ready to commence feeding 2 days after hatching (water temperature at 25 to 28°C). On the other hand, Termvidchakorn and Hortle (2013) reported the *L. hoevenii* larvae in the wild commenced exogenous feeding on zooplanktons within the first 3 days after hatching (TL 4.8 to 5.9 mm) at water temperature 26 - 29°C. These observations suggest that the yolk-sac larvae of *L. hoevenii* are capable of mixed feeding, in which they can commence the first exogenous feeding at any time during a short interval before their yolk is completely depleted (Balon, 1986). In fact, the yolk-sac larvae of many freshwater fish species are reported to be capable of mixed feeding (Jaroszewska and Dabrowski, 2011). Apparently, the suitable timing to conduct first feeding for *L. hoevenii* larvae reported by Meenakarn (1986) and Truong *et al*. (2003) are both acceptable. However, possibility for the *L. hoevenii* yolk sac larvae being capable of mixed feeding is yet to be confirmed as published information on the early larval development of *L. hoevenii* is very scarce, and the timing in conjunction of its first feeding and exhaustion of the yolk has not yet been determined. Further examination on the early larval development of *L. hoevenii* (including yolk absorption, sensory organs and alimentary tract development, and behavioural changes) in conjunction with the larval first exogenous feeding activity should be conducted in the future in order to determine the optimum first feeding timing.

Ideal food items for first feeding

There are several considerations in selecting the ideal food items for the first feeding of fish larvae. The food items' sizes and digestibility are among the main criteria (Kolkovski *et al*., 2009). According to Shirota (1970), the mouth size of larval fish at the first feeding has a close relationship to the size of their natural foods. Larval fishes with small mouth sizes usually take only phytoplanktons, protozoa, and the nauplii of small copepods, while those with big mouth sizes easily feed on the large copepods (Shirota, 1970). Therefore, in the farming practices, the larval mouth size of the target fish species should be determined so that food items of appropriate size can be provided to the larvae (Amornsakun *et al*., 2004, 2005, 2014; Tew *et al*., 2013). The digestive capability of fish larvae at the onset of first feeding is known to be limited (Lazo *et al*., 2011). The food items which are readily digestible by the fish larvae need to be identified for their first feeding (Langdon and Barrows, 2011).

At present, there is still no information on the mouth development and the digestive capability of *L. hoevenii* during its onset of active feeding. According to Meenakarn (1986) and Truong *et al*. (2003), however, boiled egg yolk, milk powder, and *Moina* sp. can be given to the *L. hoevenii* larvae for first feeding. Apparently, these food items suit the mouth sizes and can be digested by the *L. hoevenii* larvae. Indeed, these food items have been commonly used for the initial feeding of larval cyprinids (Lin and Peter, 1991). Nevertheless, development of mouth size and digestive capability in the *L. hoevenii* larvae at first feeding should be examined in order to fill in the knowledge gap vis-à-vis the larval feeding ecology of this fish.

Changes of food types with the fish growth

The growing fish larvae require food rich in nutrients and energy for their stable growth (Yúfera, 2011). It is, therefore, important to determine the suitable timing (based on the larval development stage) and food type during the process of weaning of the diets (Dabrowski, 1984; Nhu *et al*., 2010; Demir and Sarigöz, 2016). Figure 1 shows the timelines for providing the suitable food items to *L. hoevenii* during the course of its rearing obtained from the work by Meenakarn (1986) and Truong *et al*. (2003). Following their studies, co-feeding the *L. hoevenii* larvae with live feed and inert food particles (boiled egg yolk, milk powder, and *Moina* sp.) during the first 10 – 15 days of rearing are necessary. During this nursery period, the *L. hoevenii* larvae were cultured in tanks. After then, artificial feeds including fish meal, rice bran, soybean meal, wheat flour and pelleted feed can be fed to the fish when they were moved to the earthen ponds. According to Meenakarn (1986), live feeds were still necessary for the pond-cultured larvae, and can be provided to the larvae by the way of fertilizing the ponds. Indeed, Sunarno and Syamsunarno (2017) have noticed that the *L. hoevenii* larvae of 0.03 g body weight (initial fish; similar age with the 10 days old larvae in the report by Truong *et al*., 2003) had achieved the highest final body weight and specific growth rate when fed *Moina* sp. and artificial diet in the ratio 50:50, instead of feeding *Moina* sp. or artificial diet exclusively, 75% *Moina* sp. + 25% artificial diet, and 25% *Moina* sp. + 75% artificial pellet, at the end of a 45-day feeding trial. Apparently, the longer the period of co-feeding, the better the larval growth. Further study is necessary to elucidate this hypothesis, and to determine the optimum timing to switch practice from co-feeding to feeding on compound diet.

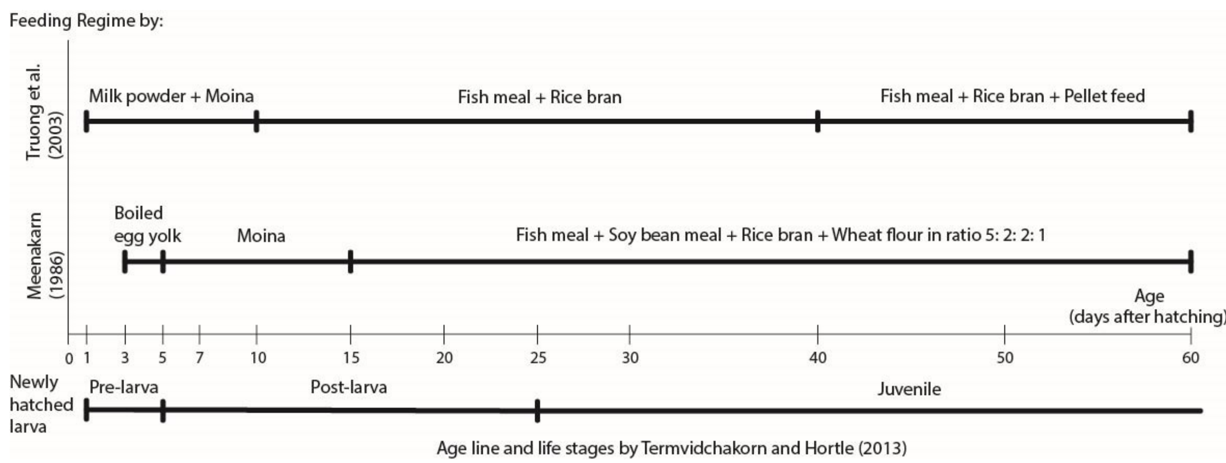

Fig. 1: The timelines of providing the suitable food items for *L. hoevenii* during the course of its rearing period, obtained from the works by Meenakarn (1986) and Truong et al. (2003).

Optimum protein requirement

Protein is one of the most important primary macro-nutrients required for fish optimum growth. It is also one of the most expensive of the ingredients in fish diet (Tacon and Metian, 2008). As dietary protein provided in excess will be catabolized into energy and contribute to more nitrogenous waste by the fish, determination of the optimum dietary protein required by the targeted fish species is important to save the feed cost and environment (Shapawi *et al*., 2014). At present, there are several studies reported on the optimum dietary protein requirement in different sizes of *L. hoevenii*. Detailed information of these studies are as shown in Table 2.

Tab. 2: Dietary protein requirement of *L. hoevenii* at different life stages.

Initial size (BW/ TL)	Feeding trial duration	Dietary protein requirement	Sources of protein included	Ref.
0.065 g	49 days	36% - 40%	Casein, soybean meal, coconut cake, corn meal, rice bran	1
1.6 g	12 weeks	30.20%	Fish meal, soybean meal, copra cake, maize, rice bran	2
2.0 g	6 weeks	29.46% - 33.76%	Casein, dextrin	3
16.5 g / 7.62 cm	168 days	38%	Poultry offal meal, palm kernel expeller, rice bran, maize, soybean meal, fish meal	4

Ref. 1: Sunarno (2002), 2: Pathmasothy and Omar (1982a), 3: Pathmasothy and Omar (1982b), 4: Farahiyah *et al*. (2017)

Through a 49 days of feeding trial, Sunarno (2002) reported that the 0.065 g of *L. hoevenii* required about 36 - 40% of dietary protein for optimum growth. On the other hand, Pathmasothy and Omar (1982a, b) demonstrated that the 1.6 g and 2.0 g *L. hoevenii* fries required about 31% of dietary protein to achieve optimum growth, through a 12-week and 6-week feeding trials, respectively. Indeed, the growing *L. hoevenii* would require higher level of dietary protein to support faster growth (National Research Council – NRC, 2011). However, Farahiyah *et al*. (2017) reported that the optimum dietary protein required by the 16.5 g of *L. hoevenii* fingerlings was 38%. This result diverges from the common fact mentioned above. According to Termvidchakorn and Hortle (2013), *L. hoevenii* only reached maturity at sizes of 500 – 600 g, hence the high dietary protein requirement reported by Farahiyah *et al*. (2017) is not for gonad maturation. Apparently, this outcome could be due to the different types of protein sources used in each of these studies (see Table 2). Unfortunately, only Sunarno (2002) provided the digestibility coefficients index of the experimental diets among these studies. Also, none of these studies has provided the amino acids content of the experimental diets. Comparison on the protein quality and utilization efficiency of the experimental feeds across these studies is, therefore, difficult to be made. However, these studies generally summarized that *L. hoevenii* would require about 30 – 40% of dietary protein for its optimum growth from larval to the grow-out stages. This requirement is similar to those recommended in the practical diets for Nile tilapia (*Oreochromis niloticus*) (FAO, 2018a) and African catfish (*Clarias gariepinus*) (FAO, 2018b) which are the most commonly cultured freshwater fish in the Southeast Asia region (SEAFDEC, 2017). Table 3 shows the comparison of the dietary protein requirement of these mentioned fish species at different life stages.

Other than protein requirement, there is still no study reported on the requirement of *L. hoevenii* for other nutrients, including lipid, vitamins, and minerals. Studies on these topics should be pursued in order to provide a comprehensive knowledge of the nutrient requirement of *L. hoevenii* for formulated feeds development.

Alternative protein sources in the diets for *L. hoevenii*

Fish meal is commonly used as the major protein source in fish diets but its price is getting expensive (Tacon and Metian, 2008). Therefore, many studies have been conducted to identify the suitable alternative protein sources to substitute or replace the fish meal in fish diets (Ayadi *et al*., 2012; Lim *et al*., 2014). Digestibility is a key parameter to determine the suitability of an ingredient as an alternative protein source for fish diets (Lim *et al*., 2014). Table 4 shows the apparent digestibility coefficients of nutrients in various ingredients in the diet of *L. hoevenii*. According to Law (1984), copra cake rather than maize, soybean, rice bran and tapioca, was the most suitable plant-based ingredient to be included in the diets for *L. hoevenii* as the fish can effectively digest most of its nutrients, except ash. Similar results were also reported by Yanto *et al*. (2017a) on the *Saccharomyces cerevisiae* fungus–fermented yellow corn meal (FYCM). Other than plant-based ingredients, terrestrial animal-based product such as the poultry offal meal (Farahiyah *et al*., 2017), was also reported to be well-digested by *L. hoevenii*. Apparently, *L. hoevenii* can easily digest both plant– and terrestrial animal– based proteins due to its omnivorous feeding habits (Roberts, 1993).

Tab. 3: Comparison on the dietary protein requirements of *L. hoevenii* with *O. niloticus* and *C. gariepinus* at different life stages.

Life stages[1] (sizes)	Dietary protein requirement		
	L. hoevenii [2]	*O. niloticus* [3]	*C. gariepinus* [4]
Fry (0.02–1.0 g)	36–40%	40%	-
Fingerlings (1.0 g–10.0 g)	29.5–33.8%	35–40%	-
Juveniles (10.0–25.0 g)	38%	30–35%	35–38%

[1] Following the definition for *O. niloticus* in FAO (2018a)
[2] References refer to Table 2
[3] Modified from FAO (2018a)
[4] Modified from FAO (2018b)

In fact, the *L. hoevenii* fed diets, partially included with either plant– or terrestrial animal– based proteins can also grow well. Table 5 shows the dietary optimum inclusion levels of these protein sources in the diets for *L. hoevenii* determined through feeding trials. Yanto *et al*. (2017a) found that including 30% of the FYCM in the diet can promote optimum growth for the *L. hoevenii*. In addition to that, supplementation of 1.55 mg kg^{-1} chromium (Cr^{3+}) into the 30% FYCM diet can further enhance the growth of the *L. hoevenii* (Yanto *et al*., 2017b). Besides, Chan *et al.* (1981) reported that the fresh poultry processing wastes can be fed directly to the *L. hoevenii* without any adverse effect on the fish growth. Farahiyah *et al.* (2017) also evidenced that *L. hoevenii* grow best when they were fed diets at 38% crude protein level with 40% inclusion level of poultry offal meal. Other than that, Yanto (2010) reported that about 30% of fish meal used in the diet for *L. hoevenii* can be replaced by the shrimp head silage meal, without affecting the fish growth performance. In summary, *L. hoevenii* can utilize various types of ingredients as the protein source in its diets. This poses an advantage for the culture of *L. hoevenii* as the low-cost ingredients can be used as dietary protein sources to reduce the feed production cost.

Dietary immuno-stimulants

Supplementation of feed additives in fish diets is sometimes necessary, especially ingredients that are immuno-stimulants, and have the quality to enhance the fish non-specific immune responses that may improve the fish survival during diseases outbreak (Gannam and Schrock, 1999; Wang *et al*., 2017). There are several studies reported on the infection of *L. hoevenii* culture (Shaharom-Harrison *et al*., 1990; Székely *et al*., 2009; Ruhil Hayati *et al*., 2015) but investigations on the immuno-stimulant supplementation for diseases prevention are very limited. At present, only Prasetio *et al*. (2017a, b) reported that the dietary supplementation of *Aloe vera* powder can enhance the survival of the *Aeromonas hydrophilla* - infected *L. hoevenii*. The optimum dietary supplementation level of *Aloe vera* powder was determined at 40 g/ kg on approximately 8 – 12 cm or 10 – 15 g of *L. hoevenii* fingerlings. Diseases outbreak can cause huge economic loss in freshwater fish culture, and hence prevention measures should receive due consideration (Bagum *et al*., 2013; Monir *et al*., 2015). Further studies to identify the suitable feed additives for diseases prevention in the *L. hoevenii* farming are highly recommended.

Tab. 4: Apparent digestibility coefficients of different sizes of *L. hoevenii* maintained on various alternative protein sources.

Initial fish sizes (BW/ TL)	Ingredients	Apparent digestibility coefficients (ADC) of the test ingredients*					Ref.
		Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Gross energy (%)	
12–26 cm	Fish meal	103.90	96.06	44.73	97.13	103.13	1
	Maize	41.85	80.41	22.69	71.47	55.87	
	Soybean	69.50	60.70	72.44	73.82	59.30	
	Copra cake	75.39	98.36	50.03	72.67	73.21	
	Rice bran	30.81	44.21	89.99	57.71	43.56	
	Tapioca	N/D	N/D	66.99	52.05	3.211	
32.54 g	*Saccharomyces cerevisiae* fungus- fermented yellow corn meal	73.28	69.46	40.20	97.33	84.77	2
16.5 g / 7.62 cm	Poultry offal meal	100.00	N/A	100.00	N/A	100.00	3
	Palm kernel expeller	74.00	N/A	57.00	N/A	60.00	
	Rice bran	78.00	N/A	83.00	N/A	84.00	

N/D = Not digested; N/A = Not available
* ADC of the test ingredient (%) = (100/ percentage of test ingredient)-((nutrient digestibility coefficient of the test diet – percentage of the reference diet)/(100 × nutrient digestibility coefficent of the reference diet))
Ref.: 1: Law (1984), 2: Yanto *et al*. (2017a), 3: Farahiyah et al. (2017)

Tab. 5: Optimum inclusion level of various alternative protein sources in the diets for different sizes of *L. hoevenii*.

Initial fish size (BW/ TL)	Feeding trial duration	Dietary protein levels	Alternative protein source used	Results	Ref.
12.74 g/8.99 cm 41.53 g/12.26 cm	90 days	N/A	Poultry processing wastes (direct feeding)	The fish can feed on the processing waste without additional preparation and grow well	1
1.11 g	50 days	37%	Shrimp head silage meal (SHSM)	SHSM can substitute 30% of the total amount of fish meal used in the diet.	2
32 .54 g	60 days	30%	Yellow corn meal (YCM) fermented with the *Saccharomyces cerevisiae* fungus	30% of fermented YCM can be included into the diet formulation	3
16.5 g/7.62 cm	168 days	38%	Poultry offal meal (POM)	40% of POM can be included into the diet formulation	4

Ref: 1: Chan *et al*. (1981), 2: Yanto (2010), 3: Yanto *et al*. (2017a), 4: Farahiyah *et al*. (2017)

Conclusion

In general, the feeding guideline for *L. hoevenii* farming has been duly developed. For feeding of *L. hoevenii* broodstock, high dietary protein diets (30 - 40%) are necessary but the optimum dietary protein and lipid required by the brood fish should be further determined. The nutritional effects of direct provision of plant ingredients to the broodstock as supplementary foods remained to be further studied as well. The practice of providing boiled egg yolk, milk powder and *Moina* sp. to the *L. hoevenii* larvae for first feeding is acceptable but the knowledge on larval development biology (including the development of larval mouth, sensory organs, digestive tract and its digestive capability) during the onset of first feeding should be investigated to understand the feeding ecology and determine the optimum first feeding timing for this fish. During the nursery period, the maximum duration of co-feeding using live and artificial feeds should be determined in order to optimize the growth and survival of the fish. From larval to juvenile stages, the optimum dietary protein requirements of *L. hoevenii* is 30 – 40%. The fry and fingerling are capable of utilizing various animal– and plant– based alternative protein sources. Nevertheless, its dietary requirements for other major nutrients including lipid, vitamins and minerals should be further studied. There is a paucity of data on the effects of feed additives for diseases prevention in the *L. hoevenii*. Further studies on this topic are highly recommended.

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