Journal of Aquaculture and Fish Health Vol. 11(3) - September 2022
DOI : 10.20473/jafh.v11i3.25879

Effect of Feeding Fermented *Leucaena leucocephala* Seed Meal on Growth and Protein Content of *Osphronemus gouramy* Juvenile

Putri Dista Ananda[1], Safrida S.[1*], Khairil K.[1], Hasanuddin H.[1] and Asiah M. D.[1]
[1]Department of Biology Education, Faculty of Teacher Training and Education, Syiah Kuala University, Jalan Tgk. Hasan Krueng Kalee, Darussalam, Banda Aceh, Aceh 23111, Indonesia

*Correspondence :
saf_rida@unsyiah.ac.id

Received : 2021-03-09
Accepted : 2022-07-29

Keywords :
Gouramy, Growth, Lamtoro, Protein content

Abstract

Gouramy (*Osphronemus gouramy* Lac.) is one of the original fish from Indonesia that lives in fresh water and has high economic value because of its dense texture, high nutrition, and delicious taste so that it is widely favored by Indonesians. The results of juvenile cultivation activities have not been going well so far, this is because the growth of gourami's juvenile is slower than the growth of other freshwater fish's juvenile. One alternative that can increase the growth and protein content of gouramy juveniles is to utilize the availability of lamtoro seeds. The purpose of this study was to determine the effect of feeding lamtoro seed fermentation on the growth and protein content of gourami's juvenile. This study used an experimental method with a completely randomized design (CRD with 6 treatments and 4 replications). Data were analyzed using the Analysis of Variance Test (ANOVA) and further tested in Honest Significant Difference (Tukey HSD Test) at the 95% confidence level. The results showed that P3 with the addition of fermented lamtoro seed flour 40% can increase the growth and protein content of gouramy juveniles. The conclusion of this study was lamtoro seed fermented feed has an effect on increasing the growth and protein content of gourami's juvenile.

INTRODUCTION

Gouramy (*Osphronemus gouramy* Lac.) is one of the original fish in Indonesian water that lives in fresh water and has high economic value because of its dense texture, high nutrition, and delicious taste it is widely favored by Indonesians. In addition, gouramy has other advantages such as being omnivorous, spawning naturally, and can live in stagnant water and at low oxygen solubility (Nugroho *et al.*, 2015). However, the constraints in intensive maintenance of gouramy are still being felt. The result of gouramy cultivation has not been going well, it is because the growth of gouramy is slower than other freshwater fish.

The success of aquaculture is determined by several factors, one of them being the availability of fish meal quality. This is because the meal is a source of protein for fish growth. Protein plays a role in body maintenance, tissue formation, and the replacement of damaged body tissue (Anshar *et al.*, 2018). However, about 60% to 85% of aquaculture production costs come from the meal. This is due to the high price of

meal raw materials, most of them still come from imports. Therefore, one of the efforts to reduce dependence on imported raw materials is by utilizing quality local raw materials. One of the local and natural ingredients that can be used as high protein fish meal is lamtoro seeds (*Leucaena leucocephala* Lam.).

Lamtoro (*L. leucocephala* Lam.) is a plant that is often found in various regions in Indonesia and has many benefits. Its abundant availability is only used by the community as a shade for roads or yards. According to research results, lamtoro seeds have high nutritional value because of their high protein content (24.5% to 46%) which contains arginine, alanine, cysteine, glutamic acid, isoleucine, leucine, lysine, and methionine (Verma *et al*., 2018). Lamtoro seeds contain anti-nutritional substances such as tannins and phytic acid which are quite high (Bakti, 2001). Anti-nutritional substances are inhibitors that can interfere with fish growth. Anti-nutritional substances contained in lamtoro seeds are very complex compounds that are difficult for fish to digest. There needs to be a special technique so that these substances can be digested properly by fish. One of the techniques used to convert anti-nutritional substances into simpler compounds is the fermentation technique.

The purpose of the fermentation process is to break down indigestible materials into easily digestible materials with the help of microorganisms. It is expected that there will be an increase in the quality of fermented meal ingredients that will be used as a mixture of fish meals to increase fish growth (Agustono *et al.*, 2012). One of the molds that can be used in the fermentation process is *Rhizopus oligosporus*. This type of mold is thought to better ferment in simplifying the anti-nutritional substances contained in plants (Putra *et al.*, 2020). In addition, economically *R. oligosporus* is easy to get at a low price and is able to develop in relatively low-cost media (Ikhwanuddin *et al.*, 2018).

The objective of this study was to determine the effect of feeding lamtoro seed fermentation on the growth and protein content of gourami's juvenile.

METHODOLOGY

Place and Time

This research was carried out from June to December 2020, this research was conducted in the Biology Education Laboratory, Chemistry Education Laboratory, and Laboratory of nutrition science and feed technology, Faculty of Agriculture Universitas Syiah Kuala Banda Aceh.

Research Materials

The main materials of this study were 72 *O. gouramy* juveniles (body length 10,5 cm and body weight 20 gram) and lamtoro seeds. The juveniles were obtained from a fish cultivator in Kota Langsa. The main equipment used in this study was 24 ponds (30x60x60 cm), scale, thermometer, pH meter, kjeldahl steam distiller, digestion apparatus, distillation flask, Erlenmeyer, and hot plate stirrer.

Research Design

The type of this research is experimental research and the research method used is an experimental method with a non-factorial Completely Randomized Design (CRD) pattern consisting of 6 treatments and 4 replications. The approach used in this research is a quantitative approach. The treatments were P0 = commercial pellet feed (100%), P1 = 0% fermented lamtoro seed flour + 80% soybean flour + 20% additives, P2 = 20% fermented lamtoro seed flour + 60% soybean flour + additional ingredients 20%), P3 = giving 40 % fermented lamtoro seed flour + 40% soybean flour + 20% additional ingredients), P4 = giving 60% fermented lamtoro seed flour + 20% soy flour + 20% additives), P5 = giving 80% fermented lamtoro seed flour + 0% soybean flour + 20% additives). The treatment was based on the preliminary test results, which

showed the highest growth of gouramy juvenile obtained by feeding fermented lamtoro seed meal by 40%, so these results were used as a reference to determine the treatment level in the study.

Work Procedure

Lamtoro Seed Flour Fermentation

The lamtoro seeds were washed, soaked for 72 hours, then dried for 48 hours under the sun, and grounded to produce flour (Pratiwy *et al*., 2020). Lamtoro seed flour was inoculated with *R. oligosporus* at a dose of 1.5 grams per 1/2 kilogram of lamtoro seed flour to be fermented. put the lamtoro seed flour in a heat-resistant plastic bag that has been perforated in several places to get aerobic conditions. Then the lamtoro seed flour is incubated at 30 °C or room temperature for 36 hours (Utami *et al.*, 2012).

Fish Rearing

The rearing of the test animals was carried out in a pond filled with water as much as 75% of the volume of the pond and given an aerator. 4 fish seeds were stocked in each pond and acclimatized for 3 days. then gouramy seeds were given treatment for 30 days. During the rearing of the gourami fry were fed according to the predetermined concentration. The frequency of application was twice a day in the morning at 08.00 WIB and in the afternoon at 17.00 WIB. Then on the 15th and 30th days the fish were measured in weight, length and body width. Water quality is also measured by measuring pH and temperature.

Measuring Protein Levels

Each sample of gouramy was slashed using a knife from the right side of the body along 1 cm from the tip of the operculum to the middle of the body of the gouramy. Samples from the results of the incision are inserted into the protein content measuring device.

Data Analysis

Data on gouramy juvenile protein content was obtained by using the Lowry method and the Kjeldahl method after 30 days. Protein content data were analyzed using a percentage formula and described. The formula for calculating the percentage of protein content is:

$$PC = \frac{0.1H_2SO_4 \times TV \times 0.014 \times 6.25 \times 5}{W} \times 100\%$$

Where:
PC = protein content (%)
TV = titration value (ml)
W = sample weight (g)

The growth and protein content data that has been analyzed was then analyzed with the Analysis of Variance Test (ANOVA) and continued with the Honest Significant Difference Test (Tukey HSD) with a confidence level of 5%.

RESULTS AND DISCUSSION

The results of this research that has been carried out for 30 days show that there are differences in the growth and protein content of gouramy juveniles from each treatment given.

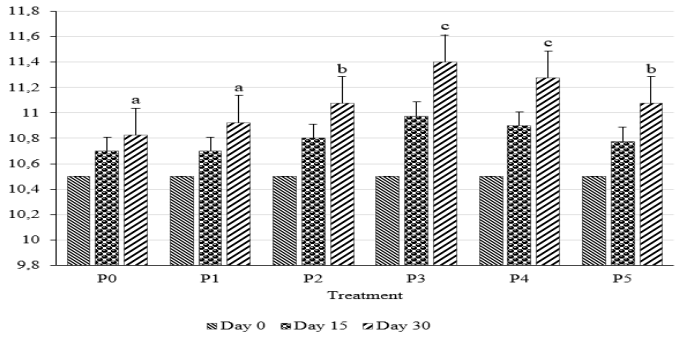

Figure 1. Average value of gouramy juvenile body length (cm).

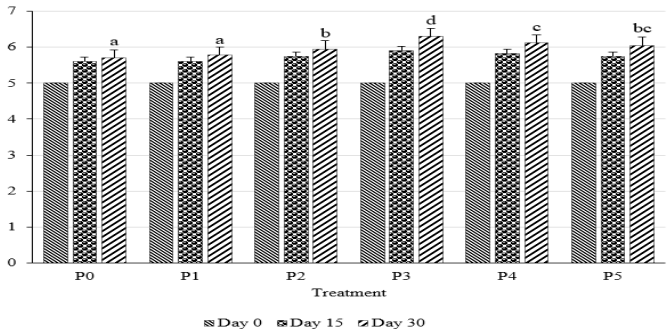

Figure 2. Average value of gouramy juvenile body width (cm).

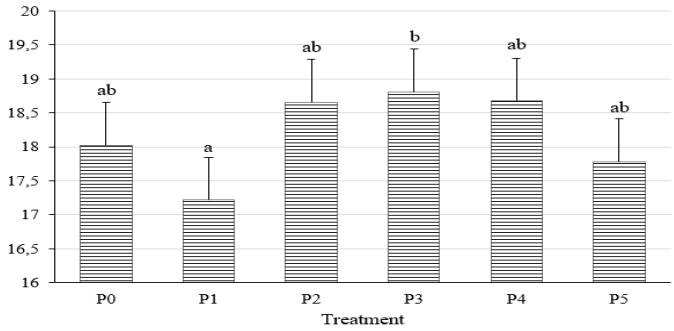

Figure 3. Average value of gouramy juvenile protein content (%).

The highest average value of gouramy juvenile's body length and width is 11,4 cm and 6,3 cm. The average value of protein content of gouramy juvenile is 17-18%. There is a significant difference in body length (Figure 1), body width (Figure 2), and protein content of gouramy juveniles given different feed doses (Figure 3).

Analysis of variance carried out on gouramy juvenile after being given different treatments for 30 days showed that F count 41.36>F table 2.77 for body length and F count 66>F table 2.77 for body width. Variance analysis data are presented in Table 1 and Table 2.

Table 1. Analysis of variants on body length of gouramy juvenile.

Source of Variation	Degrees of Free	Sum of Squares	Middle Square	F Count	F Table
Treatment	5	0,91	0,182	41,36*	2,77
Error	18	0,08	0,0044		

Table 2. Analysis of variants on body width of gouramy juvenile.

Source of Variation	Degrees of Free	Sum of Squares	Middle Square	F Count	F Table
Treatment	5	0,99	0,198	66*	2,77
Error	18	0,06	0,003		

Analysis of variance carried out on protein content of gouramy juvenile after being given different treatments for 30 days showed that F count was 3.67>F table was 2.77. Variance analysis data are presented in Table 3. To find out the differences between the treatments given, the research data were further tested with the BNJ Advanced Test (Honest Real Difference)/Tukey HSD. The results of the BNJ Advanced Test are presented in Table 4.

Table 3. Analysis of variants on protein content of gouramy juvenile.

Source of Variation	Degrees of Free	Sum of Squares	Middle Square	F Count	F Table
Treatment	5	7,54	1,508	4,496*	2,77
Error	18	7,39	0,411		

Table 4. Average of gouramy juvenile protein content (HSD 0.05).

Treatment	Average	HSD 0,05
P0	18,025	Ab
P1	17,267	A
P2	18,657	Ab
P3	18,812	B
P4	18,675	Ab
P5	17,782	Ab

Description: The numbers followed by the same letter are not significantly different from the HSD Test at the 5% level.

The results of water quality measurements that have been carried out in this study indicate that the water quality in the research pond is in good condition. The value range of water quality during the study is presented in Table 5.

Table 5. The value range of water quality.

Treatment	Temperature			pH		
	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
P0	30 °C	29 °C	30 °C	8.0	7.8	7.9
P1	30 °C	29 °C	30 °C	8.0	7.8	7.9
P2	30 °C	29 °C	30 °C	8.0	7.8	7.9
P3	30 °C	29 °C	30 °C	8.0	7.8	7.9
P4	30 °C	29 °C	30 °C	8.0	7.8	7.9
P5	30 °C	29 °C	30 °C	8.0	7.8	7.9
Average	30 °C	29 °C	30 °C	8.0	7.8	7.9

The results of this research that has been carried out for 30 days indicate that fermented lamtoro seed meal has an effect on the growth and protein content of gouramy juveniles. Fish can grow well if their nutritional needs are fulfilled, especially protein needs. Providing quality meals, both artificial meals and forage at the juvenile stage is an important thing (Juliana *et al*., 2018). Anti *et al.* (2018) explains that the protein content contained in the fish body comes from feed which is converted into protein stored in the fish's body which can be absorbed and used to build or repair damaged body cells and utilized by the fish body for metabolic processes. This is also referred to as protein retention. Furthermore, Dani *et al.* (2005) explains that the fast or slow growth of fish is determined by the amount of protein that can be absorbed and utilized by fish as a building block.

A meal is needed for growth, fish health, and increasing the quality of fish production. The complete nutritional content of the meal is always associated with the ingredients used in preparing the meal formulation. One of the important meal nutrients needed by fish is protein. Protein is a source of energy other than carbohydrates which is useful for the growth and survival of fish (Marzuqi and Anjusary, 2013). The protein contained in fermented lamtoro seed can help fish growth. The results showed that protein content in lamtoro seed flour soaked and dried in the sun increased to more than 30% (Sotolu and Faturoti, 2008). Similar results were also obtained by Sethi and Kulkarni (1993), who stated that lamtoro seeds soaked in water have 31% protein content and could reduce mimosine level up to 82.1% in 100 g/g protein.

The body protein content of *O. gouramy* juvenile is influenced by the protein content and amino acids of its meal. Gouramy juvenile that was given 0% lamtoro seed fermented meal has the lowest body protein content and was significantly different from the fish body protein content in P3 by giving lamtoro seed fermented meal by 40%. Feed protein content affects protein retention. Protein retention describes the proportion of feed protein stored as protein in fish body tissues. According to Phumee *et al.* (2009), fish body protein content is influenced by meal protein intake and protein deposits which have a positive correlation with meal protein content. This is reinforced by the results of research by Salhi *et al.* (2004) and Satpathy *et al.* (2003), that the addition of protein content in meals affects the protein content of fish bodies.

Fish is a source of protein that is needed by humans because it has a high protein content, contains essential amino acids that the body needs, and the price is much cheaper when compared to other animal protein sources (Natsir, 2018). Prameswari (2018) adds that fish protein absorption is higher than beef, chicken, and others. This is because fish meat has a shorter protein fiber than beef or chicken. According to Fridawanti (2016), protein needs in adulthood are 50-60 grams per day or around 11% and the need for animal protein in adolescents is 20%-40% (Suryandari and Widyastuti, 2015). In this study, the highest protein content of gouramy juvenile was found in juveniles fed fermented lamtoro seeds by 40% with a crude protein value of 19% per 0.7 grams of juvenile meat. So, it can be said that juveniles fed with fermented lamtoro seeds on P3 can supply the needs of animal protein for humans.

Protein is a source of essential amino acids needed by fish to support optimum growth as well as a source of energy for fish. According to Kardana *et al.* (2012), the protein requirement for omnivorous fish at the juvenile stage is 42%. Fish can grow well if their nutritional needs are fulfilled optimally, especially protein needs. According to Santoso and Agusmansyah (2011), protein is very important for the fish's body because nearly 65%-75% of the fish's dry weight is protein. Fish consume protein to obtain amino acids which will be used for the maintenance of body cells, growth, and reproduction. Asfiya *et al.* (2017) explains, in general, the need for a fish juvenile for amino acids is higher than in other phases. Lysine and leucine are the most needed amino acids compared to other amino acids. The highest level of essential amino acids contained in lamtoro seeds was arginine at 2.62% and the lowest level of essential amino acids was methionine at 0.36%. Apart from leucine and methionine, other essential amino acids contained in lamtoro seeds were leucine at 1.81%, lysine at 1.39%, and isoleucine at 0.93% (Ahmed and Abdelati, 2009).

The water temperature measured in this study ranged from 28ºC to 29ºC, which according to El-Sayed and Kawanna (2008), a temperature in the range of 24ºC to 32ºC is the optimal temperature that can increase the growth of freshwater fishes. Qiang *et al.* (2013) also stated that water temperature affects the digestibility of fish feed. Warmer temperatures can increase the consumption and utilization of fish feed so that the digestion process becomes more efficient. The pH of the water measured during this study was in the range of 7 to 8, which is the optimal pH that can increase the growth of gouramy. A similar result was also obtained by Pratama and Muqti (2018), who stated that the best pH for gourami's growth is 7,8. According to Indra *et al.* (2013), pH is a limiting factor that can influence and determine the amount of feed consumption and the metabolic rate.

CONCLUSION

Gouramy (*O. gouramy* Lac.) juvenile that was given fermented lamtoro seed (*L. leucocephala* Lam.) meal has an effect on the growth and protein content of its body. The results showed that the addition of lamtoro seed fermented meal by 40%

affected the growth and protein content of gouramy juvenile. It is recommended that further research be carried out regarding the addition of maintenance time and giving fermented lamtoro seed meal from juvenile phase to harvest period and to measure the organ health of gouramy which is fed with fermented lamtoro seed meal.

ACKNOWLEDGMENT

Writers would like to appreciate to Laboratory of Nutrition Science and Feed Technology, Faculty of Agriculture, Syiah Kuala University for their support of all research activities.

REFERENCES

Agustono, Putri, D.R. and Subekti, S., 2012. The Content of Dry Matter, Crude Fiber and Protein from Fermentation of *Leucaena glauca* Leaf Using Probiotic as Fish Feed Material. *Jurnal Ilmiah Perikanan dan Kelautan*, *4*(2), pp.160-168. https://doi.org/10.20473/jipk.v4i2.11567

Ahmed, M.E. and Abdelati, K.A., 2009. Chemical composition and amino acids profile of *Leucaena leucocephala* seeds. *International Journal of Poultry Science*, *8*(10), pp.966-970. https://doi.org/10.3923/ijps.2009.966.970

Anshar, Hamzah, M., Muskita, W.H. and Kurnia, A., 2018. Pengaruh substitusi tepung kedelai (*Glicine max*) dengan tepung daun kelor (*Moringa oleifera*, Lam) terhadap pertumbuhan dan kelangsungan hidup ikan bandeng (*Chanos chanos*). *Jurnal Sains dan Inovasi Perikanan*, *2*(2), pp.40-48. http://dx.doi.org/10.33772/jsipi.v2i2.6578

Anti, U.T., Santoso, L. and Utomo, D.S.C., 2018. Pengaruh suplementasi tepung daun kelor (*Moringa oleifera*) pada pakan terhadap performa pertumbuhan ikan gurami (*Osphronemus gouramy*). *Jurnal Sains Teknologi Akuakultur*, *2*(2), pp.22-31. http://jsta.aquasiana.org/index.php/jmai/article/view/49

Asfiya, M., Safrida and Abdullah, 2017. The Effect of Supplementary Feeding from Gold Snails (*Pomacea canaliculata* L.) on Growth of Gouramy Fish (*Osphronemus gouramy* Lac.). *Jurnal Ilmiah Mahasiswa Pendidikan Biologi*, *2*(3), pp.1-10. http://www.jim.unsyiah.ac.id/pendidikan-biologi/article/view/3484/2375

Bakti, I.A.R., 2001. Fermentasi biji lamtoro gung (*Leucaena leucocephala*) untuk menghasilkan tanin sebagai komoditi farmasi dan lain-lain. *Jurnal Penelitian Sains*, *10*, pp.103-112. https://doi.org/10.56064/jps.v0i10.357

Dani, N.P., Budiharjo, A. and Listyawati, S., 2005. Komposisi pakan buatan untuk meningkatkan pertumbuhan dan kandungan protein ikan tawes (*Puntius javanicus* Blkr.). *BioSMART: Journal of Biological Science*, *7*(2), pp.83-90.

El-Sayed, A.M. and Kawanna, M., 2008. Optimum water temperature boosts the growth performance of nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system. *Aquaculture Research*, *39*(6), pp.670-672. https://doi.org/10.1111/j.1365-2109.2008.01915.x

Fridawanti, A.P., 2016. Hubungan antara asupan energi, karbohidrat, protein, dan lemak terhadap obesitas sentral pada orang dewasa di Desa Kepuharjo, Kecamatan Cangkringan, Yogyakarta. Bachelor Thesis. Universitas Sanata Dharma. Yogyakarta.

Ikhwanuddin, M., Putra, A.N. and Mustahal, 2018. Utilization of Rice Bran Fermentation with Aspergillus niger on Feed Raw Material of Tilapia (*Oreochromis niloticus*). *Jurnal Perikanan dan Kelautan*, *8*(1), pp.79-87. http://dx.doi.org/10.33512/jpk.v8i1.3793

Indra, R.T., Iriana, D. and Herawati, T., 2013. Pengaruh pemberian pakan

alami *Tubifex* sp., *Chironomus* sp., *Moina* sp., dan *Daphnia* sp. terhadap pertumbuhan benih ikan gurame padang (*Osphronemus gouramy* Lac.). *Jurnal Perikanan dan Kelautan*, *4*(3), pp.283-290. https://pustaka.unpad.ac.id/wp-content/uploads/2015/02/14-Pengaruh-Pemberian-Pakan-Alami-Tubifex-sp.pdf

Juliana, Koniyo, Y. and Panigoro, C., 2018. Pengaruh pemberian pakan buatan menggunakan limbah kepala udang terhadap laju pertumbuhan dan kelangsungan hidup benih ikan gurame (*Osphronemus gouramy*). *Jurnal Ilmu Kelautan Kepulauan*, *1*(1), pp.30-39. http://dx.doi.org/10.33387/jikk.v1i1.679.g487

Kardana, D., Haetami, K. and Suherman, H., 2012. Efektivitas penambahan tepung maggot dalam pakan komersil terhadap pertumbuhan benih ikan bawal air tawar (*Colossoma macropomum*). *Jurnal Perikanan dan Kelautan*, *3*(4), pp.177-184. https://jurnal.unpad.ac.id/jpk/article/view/2560/2318

Marzuqi, M. and Anjusary, D.N., 2013. Nutrient Digestibility Feed With Different Levels of Protein and Lipid on Coral Rock Grouper (*Epinephelus corallicola*) Juvenile. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, *5*(2), pp.311-323. https://doi.org/10.29244/jitkt.v5i2.7560

Natsir, N.A., 2018. Analisis kandungan protein total ikan kakap merah dan ikan kerapu bebek. *BIOSEL (Biology Science and Education): Jurnal Penelitian Science dan Pendidikan*, *7*(1), pp.49-55. http://dx.doi.org/10.33477/bs.v7i1.392

Nugroho, I.I., Subandiyono and Herawati, V.E., 2015. Tingkat pemanfaatan *Artemia* sp. beku, *Artemia* sp. awetan dan cacing sutera untuk pertumbuhan dan kelangsungan hidup larva gurami (*Osphronemus gouramy,* Lac.). *Journal of Aquaculture Management and Technology*, *4*(2), pp.117-124. https://ejournal3.undip.ac.id/index.php/jamt/article/view/8592

Phumee, P., Hashim, R., Aliyu-Paiko, M. and Shu-Chien, A.C., 2009. Effects of dietary protein and lipid content on growth performance and biological indices of iridescent shark (*Pangasius hypophthalmus*, Sauvage 1878) Fry. *Aquaculture Research*, *40*(4), pp.456-463. https://doi.org/10.1111/j.1365-2109.2008.02116.x

Prameswari, G.N., 2018. Promosi gizi terhadap sikap gemar makan ikan pada anak usia sekolah. *Journal of Health Education*, *3*(1), pp.1-6. https://doi.org/10.15294/jhe.v3i1.18379

Pratama, N.A. and Muqti, A.T., 2018. Pembesaran larva ikan gurami *Osphronemus gouramy* secara intensif di Sheva Fish Boyolali, Jawa Tengah. *Jurnal of Aquaculture and Fish Health*, *7*(3), pp.102-110. https://doi.org/10.20473/jafh.v7i3.11258

Pratiwy, F.M., Kurniasih, T., Iskandar and Dhahiyat, Y., 2020. Replacement of soybean meal with *Leucaena* leaf meal fermented by proteolytic bacteria in diets of nile tilapia (*Oreochromis niloticus*). *International Journal of Fisheries and Aquatic Studies*, *8*(1), pp.160-163. https://www.fisheriesjournal.com/archives/2020/vol8issue1/PartC/8-1-15-924.pdf

Putra, A.N., Maula, I.M., Aryati, Syamsunarno, M.B. and Mustahal, 2020. Evaluasi tepung daun kelor (*Moringa oleifera*) yang dihidrolisis cairan rumen domba sebagai bahan baku pakan ikan lele (*Clarias* sp.). *Jurnal Perikanan Universitas Gadjah Mada*, *22*(2), pp.133-140. https://doi.org/10.22146/jfs.57468

Qiang, J., Wang, H., Kpundeh, M.D., He, J. and Xu, P., 2013. Effect of water temperature, salinity, and their interaction on growth, plasma osmolality, and gill Na^+, K^+-ATPase activity in juvenile GIFT tilapia *Oreochromis niloticus* (L.). *Journal of*

Thermal Biology, *38*(6), pp.331-338. https://doi.org/10.1016/j.jtherbio.2013.04.002

Salhi, M., Bessonart, M., Chediak, G., Bellagamba, M. and Carnevia, D., 2004. Growth, feed utilization, and body composition of black catfish, *Rhamdia quelen*, fry fed diets containing different protein and energy levels. *Aquaculture*, *231*(1-4), pp.435-444. https://doi.org/10.1016/j.aquaculture.2003.08.006

Santoso, L. and Agusmansyah, H., 2011. Pengaruh substitusi tepung kedelai dengan tepung biji karet pada pakan buatan terhadap pertumbuhan ikan bawal air tawar (*Colossoma macropomum*). *Berkala Perikanan Terubuk*, *39*(2), pp.41-50. http://dx.doi.org/10.31258/terubuk.39.2.%25p

Satpathy, B.B., Mukherjee, D. and Ray, A.K., 2003. Effects of dietary protein and lipid levels on growth, feed conversion and body composition in rohu, *Labeo rohita* (Hamilton), fingerlings. *Aquaculture Nutrition*, *9*(1), pp.17-24. https://doi.org/10.1046/j.1365-2095.2003.00223.x

Sethi, P. and Kulkarni, P.R., 1993. Fractionation of *Leucaena* seed-kernel proteins based on their solubility characteristics. *Food Chemistry Journal*, *48*(2), pp.173-177. https://doi.org/10.1016/0308-8146(93)90053-I

Sotolu, A.O. and Faturoti, E.O., 2008. Digestibility and nutritional values of differently processed *Leucaena leucocephala* (Lam. de Wit) seed meals in the diet of African Catfish (*Clarias gariepinus*). *Middle-East Journal of Scientific Research*, *3*(4), pp.190-199. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.488.593&rep=rep1&type=pdf

Suryandari, B.D. and Widyastuti, N., 2015. Hubungan asupan protein dengan obesitas pada remaja. *Journal of Nutrition College*, *4*(4), pp.492-498. https://doi.org/10.14710/jnc.v4i4.10153

Utami, I.K., Haetami, K. and Rosidah, 2012. Pengaruh penggunaan tepung daun turi hasil fermentasi dalam pakan buatan terhadap pertumbuhan benih bawal air tawar (*Colossoma macropomum* Cuvier). *Jurnal Perikanan dan Kelautan*, *3*(4), pp.191-199. http://journal.unpad.ac.id/jpk/article/view/2562

Verma, V.K., Rani, K.V., Kumar, S.R. and Prakash, O., 2018. *Leucaena leucocephala* pod seed protein as an alternate to animal protein in fish feed and evaluation of its role to fight against infection caused by *Vibrio harveyi* and *Pseudomonas aeruginosa*. *Fish & Shellfish Immunology*, *76*, pp.324-332. https://doi.org/10.1016/j.fsi.2018.03.011