Application of Rice Field Snails (Pila ampullacea) Extract as an Alternative Substitute for Protein Sources in Export-Quality Catfish Feed (Clarias sp.)

Syahruddin Kasim, Yusafir Hala, and Lulu Sri Rahayu*

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Jalan Perintis Kemerdekaan, KM. 10, Makassar, Indonesia

*Corresponding Author: kasimsyahruddin@gmail.com

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Abstract

The freshwater fishery commodity that ranks at the top in total production in Indonesia, and its high domestic consumption, is catfish (Clarias sp.). One of the efforts to provide alternative feeds is by using rice field snails (Pila ampullacea) due to their high protein content. This study aims to determine the content of P. ampullacea feed and its application as an alternative protein source in export-quality Clarias sp. The feed was prepared by combining P. ampullacea flour, rice bran, and corn flour in a ratio of 3:1:1. Analysis of water content was conducted using the weight reduction method, ash content using the ashing method, protein content using the Kjeldahl method, and fat content using the Soxhletation method. The analysis results showed that P. ampullacea had water content of 7.97%, ash content of 10.36%, protein content of 50.17%, and fat content of 4.16%. Meanwhile, the P. ampullacea feed had water content of 10.49%, ash content of 8.26%, protein content of 48.74%, and fat content of 3.6%. The results indicated that the application of P. ampullacea to Clarias sp. led to an increase in protein and fat content every week, the levels found in the commercial feed used.

Keywords: Clarias sp., P. ampullacea, feed, protein, fat

INTRODUCTION

The fisheries sector in Indonesia has immense potential, including capture fisheries resources, marine aquaculture, and public waters, with an estimated value of US$ 82 billion per year. The freshwater aquaculture sector, particularly in freshwater fish, is experiencing exponential growth in terms of production due to the relatively affordable prices for the community. Indonesian freshwater aquaculture plays a crucial role in meeting domestic and foreign fish consumption (Syamsunarno and Sunarno, 2016). Catfish, specifically Clarias sp., ranked first in freshwater fish production in 2015, with a total consumption of 82,030 tons.

Clarias sp. is a freshwater fishery commodity that contributes more than 10% to aquaculture production and has a growth rate of 17%-18%, supporting the development of fishery production in Indonesia. According to Ubadillah and Wikanastri (2010), Clarias sp. is popular among the public due to its fast growth, adaptability to the environment, good taste, and relatively low price. The nutritional composition of Clarias sp. includes protein (17.7%), fat (4.8%), minerals (1.2%), and water (76%).

According to the Central Statistics Agency (CSA), the export value of fishery products reached USD 4.94 billion by December 2019. In international marketing, exporters face challenges related to different standards and rules imposed by importing countries to ensure that fish and fishery products meet food safety and quality requirements (Pramono et al., 2014). According to the United States Department of Agriculture (2016), Clarias sp. export quality requires a protein content of 18% and a fat content of 7%.

One of the strategies to produce Clarias sp. with high protein and low fat content is through the modification of feed with high nutritional value. However, the high cost of feed production, which can reach 60-70% of the overall production cost, poses a challenge. Commercial fish feed available in the market is relatively expensive, making it necessary to explore alternative solutions, such as preparing homemade feed using inexpensive raw materials with good nutritional value (Angrgraeni and Rahmiati, 2016). One of the alternative protein sources is rice field snails (P. ampullacea), which have relatively high nutritional content. According to Megawati’s research (2020), P. ampullacea contains 39% protein,
2.93% fat, 8.69% water content, and 10.75% ash content.

Based on the information provided, research has been conducted to produce export-quality feed for *Clarias* sp. by utilizing *P. ampullacea* extract as an alternative component to substitute protein sources. The objective of this research is to provide information on the availability of high-quality feed for *Clarias* sp., enabling the production of export-quality *Clarias* sp. with high protein content and low fat content.

**METHODOLOGY**

**Instrumentals and materials**

The ingredients used were *P. ampullacea*, *Clarias* sp. seeds, rice bran flour, corn flour, commercial feed "Hi-Pro-VITE FF-999", Kjeldahl tablets, 40% NaOH, 3% H$_2$BO$_3$, concentrated H$_2$SO$_4$, Bromcresol Green indicator, Methyl Red indicator, 95% alcohol, 0.1 N HCl, n-hexane, distilled water, salt, and Whatman Sheet filter paper. The equipment used is a disc mill 45 stainless machine located at the Ujung Pandang State Polytechnic, porcelain dish, petri dish, Barmstead 6000 furnace, Spnisosfd model oven, hotplate, desiccator, ruler, various glass equipment in the laboratory. Kjeldahl flask, Ohaus digital balance, Soxhlet set, fat flask and distillation set.

**Sample preparation of *P. ampullacea***

Fresh samples of *P. ampullacea* were soaked in saltwater for 30 minutes and then drained. Furthermore, the meat of the *P. ampullacea* sample was separated from the shell and dried. The dried *P.ampullacea* meat was mashed using a grinding machine to become flour.

**Sample preparation of *Clarias* sp.**

*Clarias* sp. was obtained by taking three fish, and each fish was placed in a 500 mL beaker filled with saltwater. The fish were left in the beakers until they died. Afterwards, the *Clarias* sp. were weighed using a digital balance and their length was measured using a ruler. The heads and fins of the *Clarias* sp. were removed, and then the meat was separated from the bones.

**Measurement of water content**

The measurement of water content was carried out using the weight reduction method. The cup to be used was pre-dried for 30 minutes in an oven at a temperature of 100-105°C. The crucible was cooled in a desiccator to remove moisture and weighed (A). The sample, weighing 2 gram (B), was then wrapped in a thimble, covered with fat-free cotton, and placed into a Soxhlet connected to the fat flask. 150 mL of n-hexane solvent was poured, and extraction was carried out for 5-6 hours or until the fat solvent that fell into the fat flask was clear. The used fat solvent was evaporated with an evaporator. The fat extract in the fat flask was dried in an oven at 100-105°C for one hour. The fat flask was cooled in a desiccator and weighed (C). The

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drying stage of the fat flask was repeated until a constant weight was obtained.

**Analysis of feed potential**

The feed was prepared by mixing *P. ampullacea* flour, rice bran, and corn flour in a ratio of 3:1:1. Then, the water content, ash content, protein contents were measured according to the procedure described above.

**Applications of *P. ampullacea* feed and preparation of the maintenance container**

The container used for the maintenance of *Clarias sp.* was a bucket measuring 80 L. The container was filled with water mixed with natural rearing pond water or natural habitat water to a height of 50 cm and placed in an open space.

**Fish care and growth observation**

The *Clarias sp.* seeds used in this study were approximately 40 days old. Then, 70 seeds were stocked in rearing containers. At the beginning of the maintenance, *Clarias sp.* adapted to the new environment. The weight, length, protein content, and fat content of the *Clarias sp.* were measured (week zero). *P. ampullacea* feed was given twice a day for 7 weeks at 10:00 and 16:00. The feeding rate was 5% of the maximum total biomass in each bucket. As a comparison, the common feed used for *Clarias sp.* was VITE FF-999 in different buckets. Every week, three *Clarias sp.* were taken and their weight and length were measured to observe their growth. Then, protein content and fat content were also measured.

**RESULTS AND DISCUSSION**

**Sample preparation result**

One bucket of 40 L of *P. ampullacea* samples produced ±650 grams of *P. ampullacea* flour. The results of sample preparation *Clarias sp.* obtained an average initial length of 6.67 cm and an average initial weight of 2.15 grams. The water content analysis of *P. ampullacea* flour, *P. ampullacea* feed, and VITE FF-999 feed are shown in Figure 1.

Based on the data analysis in Figure 1, it is shown that the moisture content in the sample of *P. ampullacea* flour is 7.97%. This result is lower than the result obtained by Megawati (2020), which is 8.69%. This difference is influenced by the storage time and sample drying time (Karim, 2015). The water content of the sample will decrease with an increasing drying time (Achaglinkame et al., 2019). On the other hand, the *P. ampullacea* feed obtained a water content of 10.49%. The water content meets the standards set by the 2006 Indonesian National Standard in Table 3, which has a maximum limit of 12%. Additionally, the water content also conforms to the international standards recommended by A Practical Guide to Nutrition, Feeds, and Feeding of Catfish (Robinson et al., 2001), which have a maximum range of 10-12%. The water content for VITE FF-999 feed is 10.53%.

The moisture content of the material greatly affects the microbiological activity that can cause product damage during the storage period. If the feed contains a lot of water, the resulting feed will be moist and susceptible to mold (Hariyoko et al., 2018) because the fungus grows optimally at a moisture content of 15-20% with a temperature of 30-32 °C. Therefore, by reducing the water content, the growth of fungi in the feed can be prevented, and the storage time of the feed can be prolonged (Hendrawati, 2011).

**Measurement of ash content**

The results of the measurement of ash content in samples of *P. ampullacea* flour, *P. ampullacea* feed, and VITE FF-999 commercial feed can be seen in Figure 2. Based on Figure 2, the measured ash content in the *P. ampullacea* flour sample was 10.36%, while in the *P. ampullacea* feed it was 8.26%. This result is lower than that obtained by Megawati (2020), which is 10.75%. The ash content value indicates the amount of minerals present in the sample. High ash content can be caused by the diverse food sources digested by *P. ampullacea* in their environment and incomplete washing (Engmann et al., 2013). The ash content meets the standards set by the 2006 Indonesian National Standard for fish feed, which has a maximum limit of 15%, and also complies with the ash content standard recommended in feed by A Practical Guide to Nutrition, Feeds, and Feeding of Catfish USA (Robinson et al., 2001), which has a maximum range of 17%. The ash content for the VITE FF-999 feed was 11.53%.
The ash content significantly affects the digestibility and growth of fish. In good fish feed, the ash content should be less than 12%. A lower ash content value is desirable to ensure undisturbed fish digestibility and optimal fish growth (Setyono, 2012).

**Measurement of protein content**

The results of measuring protein content in *P. ampullacea* flour, *P. ampullacea* feed, and VITE FF-999 feed can be seen in Figure 3. Based on Figure 3, the measured protein content in *P. ampullacea* flour samples was 50.17%. According to Megawati’s research (2020), the protein content of *P. ampullacea* flour was 39%. The protein content measured in *P. ampullacea* feed was 48.74%. This aligns with the 2006 Indonesian National Standard for fish feed, which has a minimum requirement of 30%, and also complies with the standards recommended by A Practical Guide to Nutrition, Feeds, and Feeding of Catfish (Robinson et al., 2001), which range from a minimum of 26-32%. Meanwhile, the analysis on VITE FF-999 feed resulted in a protein content of 32.31%. According to Taunu et al. (2019), the VITE FF-999 feed has a protein content of 38%, and according to Oktasari (2014) in Table 4, the protein content in *P. ampullacea* is 15%.
The data significantly differs from the results obtained, indicating variations in nutritional content influenced by internal and external factors. Internal factors include the type or species of snails, sex, age, and reproductive phase, while external factors encompass the snail’s habitat and water quality in the environment where P. ampullacea lives. Snail habitat affects the nutritional content of the meat (Annisa et al., 2017).

**Measurement of fat content**

The results of the fat content analysis of P. ampullacea flour, P. ampullacea feed, and VITE FF-999 feed can be seen in Figure 4. Based on Figure 4, the measured fat content in P. ampullacea flour samples was 4.16%, and the fat content in P. ampullacea feeds was 3.6%. In Megawati’s research (2020), the fat content in P. ampullacea feed was found to be 2.93%. This result complies with the 2006 Indonesian National Standard for fish feed, which sets a maximum limit of 5% for fat content, and it also aligns with the standards recommended by A Practical Guide to Nutrition, Feeds, and Feeding of Catfish (Robinson et al., 2001), which specify a maximum range of 4-6%. The commercially available feed, particularly the VITE FF-999 feed, has a fat content of 4.76%.

According to Taunu et al. (2019), the VITE FF-999 feed has a minimum fat content of 4%, and according to Oktasari (2014) in Table 4, the fat content in P. ampullacea is 2.4%. These data differ from the obtained results due to the inclusion of additional components in P. ampullacea feed, such as rice bran with a fat content of 8.64% and corn flour.
Table 1. Growth data of *Clarias* sp.

<table>
<thead>
<tr>
<th>Feeds</th>
<th>VITE FF-999</th>
<th><em>P. ampullacea</em></th>
<th>VITE FF-999</th>
<th><em>P. ampullacea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.67</td>
<td>6.67</td>
<td>2.15</td>
<td>2.15</td>
</tr>
<tr>
<td>1</td>
<td>7.03</td>
<td>7</td>
<td>2.21</td>
<td>2.19</td>
</tr>
<tr>
<td>2</td>
<td>8.03</td>
<td>7.8</td>
<td>3.74</td>
<td>3.29</td>
</tr>
<tr>
<td>3</td>
<td>9.67</td>
<td>8.53</td>
<td>5.76</td>
<td>4.31</td>
</tr>
<tr>
<td>4</td>
<td>10.9</td>
<td>9.16</td>
<td>7.14</td>
<td>6.27</td>
</tr>
<tr>
<td>5</td>
<td>11.13</td>
<td>11.5</td>
<td>8.75</td>
<td>11.2</td>
</tr>
<tr>
<td>6</td>
<td>11.73</td>
<td>11.8</td>
<td>11.01</td>
<td>13.54</td>
</tr>
<tr>
<td>7</td>
<td>13.7</td>
<td>13.5</td>
<td>17.33</td>
<td>15.51</td>
</tr>
</tbody>
</table>

with 7.77% fat content (Dauntasik, 2019; Mukhlishah, 2019).

Fat serves as a source of energy needed by fish for their daily activities (Munisa et al., 2015). Besides being an energy source, fat also affects the storage process. Inappropriate fats can cause food to become rancid easily, decrease appetite, and lead to fish poisoning. When the fat content in the feed is appropriate, fish tend to grow faster, exhibit better feed efficiency, and reduce the risk of mortality (Prasetyowati, 2016; Hendrawati, 2011).

### *P. ampullacea* feed application on *Clarias* sp. growth.

The results showed that different feed formulations in the feed would affect the increase in length and body weight of *Clarias* sp. (Table 1). Table 1 presents the average length growth of *Clarias* sp. that were fed with VITE FF-999 and *P. ampullacea* for 7 weeks, and it indicates that the two types of feed had different effects on length growth. The percentage increase in average weight for *Clarias* sp. fed with VITE FF-999 feed was 31.96%, while for those fed with *P. ampullacea* feed, it was 30.32%. These results demonstrate that the growth of *Clarias* sp. each week yielded quite positive outcomes when fed with both VITE FF-999 and *P. ampullacea* feed. The growth rate of *Clarias* sp. carried out for 7 weeks shows in Figure 5.

Figure 5 illustrates the growth rate of *Clarias* sp. over a period of 7 weeks. The average length of *Clarias* sp. increased each week when fed with both VITE FF-999 and *P. ampullacea* feed. However, *Clarias* sp. exhibited slightly greater length growth with VITE FF-999 feed compared to *P. ampullacea* feed. The percentage increase in length for *Clarias* sp. was highest with VITE FF-999 feed in the 3rd week at 20.42% and lowest in the 5th week at 2.11%. For *Clarias* sp. fed with *P. ampullacea* feed, the highest percentage increase in length occurred in the 5th week at 25.54% and the lowest in the 6th week at 2.60%. Based on these findings, it can be concluded that *Clarias* sp. fed with *P. ampullacea* experienced slower growth in length. The slow increase in length could be attributed to prolong fish adaptation, limited digestion ability of the incoming food, and potential differences in nutrient content, particularly the protein content in the feed (Saragih, 2018).

Moreover, Figure 5 indicates that the predicted average length of *Clarias* sp. fed with *P. ampullacea* feed was nearly identical to the predicted length of *Clarias* sp. fed with VITE FF-999 feed at harvest time, specifically in the 15th week. The weight of *Clarias* sp. increased after being fed with VITE FF-999 and *P. ampullacea* for seven weeks show in Figure 6.

Based on Figure 6, it is evident that there was a significant increase in the weight of *Clarias* sp. after being fed with VITE FF-999 and *P. ampullacea* for 7 weeks. The initial weight of *Clarias* sp. fed with VITE FF-999 was 2.15 gram, which increased to 17.33 g in the 7th week. Similarly, the initial weight of *Clarias* sp. fed with *P. ampullacea* was 2.15 gram, and it reached 15.51 gram in the 7th week. The highest percentage of weight gain in *Clarias* sp. fed with VITE FF-999 was 57.40% in the 7th week, while the
lowest was 2.79% in the first week. On the other hand, the highest percentage of weight gain in *Clarias* sp. fed with *P. ampullacea* was 78.62% in the 5th week, and the lowest was 1.86% in the first week.

![Figure 6. Clarias sp. average weight graph.](image)

Based on these data, the weight increase in *Clarias* sp. fed with VITE FF-999 exhibited fluctuations from the 7th week to the 8th week. According to Fujaya (1999), fish growth is influenced by two factors: internal factors and external factors. Internal factors include heredity, gender, age, parasites, and disease, while external factors include the quality of food and water in the rearing environment. Figure 6 data indicates that the predicted average weight of *Clarias* sp. fed with *P. ampullacea* was higher than the predicted weight of *Clarias* sp. fed with VITE FF-999. Therefore, it can be concluded that the weight of *Clarias* sp. fed with *P. ampullacea* was heavier on average than the weight of *Clarias* sp. fed with VITE FF-999 at harvest.

![Figure 7. Graph of average protein content of Clarias sp.](image)

The availability of protein in fish feed is a crucial nutrient that significantly influences fish growth. Insufficient protein in the feed can result in stunted fish growth (Sukadi, 2003). Table 2 shows the results of a 7-week analysis on the protein levels in *Clarias* sp. Table 2 shows the results of measuring the protein content of *Clarias* sp. over a period of 7 weeks. In the 7th week, the protein content of *Clarias* sp. fed with VITE FF-999 was 9.35%, while the protein content of those fed with *P. ampullacea* was 10.87%. These differences can be attributed to the protein content of VITE FF-999 feed, which was 32.31%, and the protein content of *P. ampullacea* feed, which was 48.74%.

Based on the data shown in Figure 7, there is an upward trend in the protein content of Clarias sp. over the course of 7 weeks. The highest percentage increase in protein content was observed in *Clarias* sp. fed with VITE FF-999, which occurred in the 6th week with a value of 17.62%. On the other hand, the lowest percentage increase in protein content for the VITE FF-999 feed was in the 5th week at 3.17%. Similarly, for *Clarias* sp. fed with *P. ampullacea*, the highest percentage increase in protein content was observed in the 6th week at 25.31%, while the lowest was in the 2nd week at 3.75%. According to Ambia et al. (2015), these variations in protein content are influenced by various factors. Internal factors include genetic characteristics and physiological conditions of the fish, while external factors encompass feeding practices and the environment.

**Table 2. Protein data of Clarias sp.**

<table>
<thead>
<tr>
<th>Feeds</th>
<th>The protein of Clarias sp. (%)</th>
<th>Protein levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VITE FF-999</td>
<td><em>P. ampullacea</em></td>
</tr>
<tr>
<td>0</td>
<td>4.94</td>
<td>4.94</td>
</tr>
<tr>
<td>1</td>
<td>5.62</td>
<td>5.86</td>
</tr>
<tr>
<td>2</td>
<td>6.25</td>
<td>6.08</td>
</tr>
<tr>
<td>3</td>
<td>6.67</td>
<td>6.49</td>
</tr>
<tr>
<td>4</td>
<td>6.93</td>
<td>6.87</td>
</tr>
<tr>
<td>5</td>
<td>7.15</td>
<td>7.23</td>
</tr>
<tr>
<td>6</td>
<td>8.41</td>
<td>9.09</td>
</tr>
<tr>
<td>7</td>
<td>9.35</td>
<td>10.87</td>
</tr>
</tbody>
</table>

Based on the provided data, it can be inferred that the protein content in *P. ampullacea* feed may contribute to faster protein production compared to VITE FF-999 feed. However, the growth of fish is ultimately determined by the amount of protein that can be absorbed and utilized by their bodies as a building material. Therefore, for normal fish growth, the provided feed must contain sufficient energy to meet metabolic needs (Hendrawati, 2011), as well as an adequate amount of protein to facilitate the formation of new tissues for growth and repair of damaged tissues (Prihadi, 2007).
Protein quality can be assessed based on various factors, including the amino acid composition of different feed ingredients, digestibility, and the balance between protein and energy. Animal protein generally has higher nutritional content compared to plant-based protein due to its essential amino acid profile and better digestibility for Clarias sp. (Robinson and Li, 2007). According to the data presented in Figure 7, the average predicted protein content of Clarias sp. fed with P. ampullacea feed was higher than the predicted average protein content of those fed with VITE FF-999 feed until the harvest time, specifically on the 15th week. This prediction is based on the equations $y=0.732x+4.6167$ for P. ampullacea feed and $y=0.5688x+4.9242$ for VITE FF-999 feed. These predictive statistical data provide insights into the protein content in Clarias sp. until the 15th week. Based on these findings, it can be concluded that P. ampullacea feed offers good protein value for Clarias sp.

Fat content of Clarias sp.

The results of the fat content analysis in Clarias sp. with P. ampullacea and VITE FF-999 feed as shown in Table 3.

Table 3. Fat data of Clarias sp.  

<table>
<thead>
<tr>
<th>Fat of Clarias sp. (%)</th>
<th>Fat levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeds</td>
<td>VITE FF-999</td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>1</td>
<td>0.46</td>
</tr>
<tr>
<td>2</td>
<td>0.67</td>
</tr>
<tr>
<td>3</td>
<td>0.85</td>
</tr>
<tr>
<td>4</td>
<td>1.09</td>
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<td>5</td>
<td>1.24</td>
</tr>
<tr>
<td>6</td>
<td>1.39</td>
</tr>
<tr>
<td>7</td>
<td>1.43</td>
</tr>
</tbody>
</table>

Table 3 displays the fat content of Clarias sp. that were fed with VITE FF-999 and P. ampullacea, showing a significant increase over time. The highest percentage increase in fat content for Clarias sp. fed with VITE FF-999 was observed in the first week at 53.33%, while the lowest increase occurred in the seventh week at 2.87%. Similarly, for Clarias sp. fed with P. ampullacea, the highest fat content was recorded in the second week at 40%, while the lowest was in the sixth week at 6.60%. Fat serves as a source of energy for fish, supporting activities that maintain their stamina, and plays a vital role in maintaining the structure and function of cell membranes and tissues in fish organs (Edo et al., 2019).

Figure 8 depicts a graph illustrating the increase in fat content in Clarias sp. over a span of 7 weeks. In the first week, the fat content of Clarias sp. fed with VITE FF-999 increased by 0.3%, reaching 1.43% in the seventh week. On the other hand, Clarias sp. fed with P. ampullacea exhibited a fat content of 0.3% in the first week and 1.31% in the seventh week. This disparity can be attributed to the varying fat content in the respective feeds, with VITE FF-999 containing 4.76% fat and P. ampullacea containing 3.60% fat. Consequently, it can be concluded that the fat content in Clarias sp. fed with VITE FF-999 was higher compared to those fed with P. ampullacea. It is important to note that the fat content should be optimal but not excessive, as an excessive fat content in the feed may lead to oxidation during meals and result in fat accumulation in the fish’s intestines, liver, or kidneys, causing overweight issues and reduced appetite (Edo et al., 2019). The equation $y = 0.1535x + 0.2367$ in Figure 8 demonstrates the predicted average fat increase in Clarias sp. with P. ampullacea feed on a weekly basis until the time of harvest. The fat content in Clarias sp. with P. ampullacea feed does not surpass the recommended limit of 5% (SNI, 2006).

CONCLUSIONS

Based on the results of the research that has been conducted, it can be concluded that the protein content of P. ampullacea and P. ampullacea feeds respectively were 50.17% and 48.74%, while the fat content respectively was 4.16% and 3.60%. The protein content of P. ampullacea and P. ampullacea feeds met the requirements of the Indonesian National Standard for Clarias sp. export quality, which is at least 26-32%, as well as the fat content, which should be maximum 5%. The protein and fat content of Clarias sp., which had been included in the P. ampullacea feed, had a positive impact on the growth

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of *Clarias sp.*, as evidenced by the increase in weight, length, protein content, and fat content every week over a span of 7 weeks.

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