Effect of carbohydrate types on water quality, proximate composition and glycogen content of white shrimp juvenile

*Litopenaeus vannamei (Boone, 1931)*

Zainuddin¹, S Aslamyah¹ and Hadijah²

¹Department of Fisheries, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia
²Department of Fisheries, Faculty of Agriculture, University of Bosowa, Makassar, Indonesia

Email: zainuddinlatief@gmail.com

**Abstract.** Vannamei shrimp is one type of penaeid shrimp that has fast growth, resistance to disease and tolerance to changes in water quality. In farming vannamei shrimp, feed is an important factor because the largest production cost comes from the purchase of feed. One way to reduce feed costs is to reduce the protein component and increase the carbohydrate component in feed formulation. This study aimed to examine the effects of various types of carbohydrates on water quality, proximate composition and glycogen content of juvenile vannamei shrimp. The study was designed with a complete randomized design with four treatments and three replicates. The carbohydrate treatments consisted of A. corn flour, B. sweet potato flour, C. rice bran flour and D. wheat flour. The shrimp seed used in this study were stadia PL25 juvenile vannamei shrimp. The feed was given at a rate of 10% of shrimp body weight with a frequency of four times a day. The results showed that the type of carbohydrate in the feed had significant effect on water quality, proximate composition and vannamei shrimp glycogen levels. Carbohydrate from sweet potato flour gave the best results compared with the other treatments.

1. **Introduction**

The vannamei shrimp *Litopenaeus vannamei* (Boone, 1931) is a penaeid shrimp that has become popular in aquaculture due to its relatively fast growth, resistance to disease and tolerance to changes in water quality. In farming vannamei shrimp, feed is an important factor because the purchase of feed is usually the largest production cost. One effort being made to increase the production of vannamei shrimp in Indonesia is the provision of quality shrimp feed affordable to pond farmers. Quality shrimp feed components include protein, fat, carbohydrates, vitamins and minerals. Protein is a nutritional component that is needed to repair tissue damage and maintain routine functions of the shrimp body [1]. High protein in feed formulation is able to accelerate the growth of vannamei shrimp [2]. However, high protein in feed will have an impact on high feed prices, potentially pollute the environment due to nutrient rich shrimp faeces, and does not support the development of sustainable shrimp culture [1].

Research on nutrition in aquaculture has shown that increasing non-protein components (e.g. fat and carbohydrates) is one strategy which can be used to minimize the use of protein in feed [3].
Although shrimp have limited ability to utilize carbohydrates and cannot adapt to high-level carbohydrates [4], carbohydrate components are often included in artificial feed for crustaceans as an energy source through the protein-sparing effect mechanism [1,2]. A number of researchers have found that penaeid shrimp species have limited ability to digest glucose, including the white shrimp *L. vannamei* [5]. The results of a previous study [6] showed that, with a feeding frequency of four times per day, *vannamei* shrimp feed formulations with a relatively high carbohydrate content of 38% gave a higher growth rate than feed with lower carbohydrate contents of 18% and 32%. In addition, it has been found that carbohydrates can meet the high energy needs of aquatic animals under stress conditions [7] because carbohydrates are the main energy source which is directly used by most crustacean species (Lehninger 1978 in [1]).

The use of carbohydrates in *vannamei* shrimp feed is now common. In general, studies on shrimp growth and carbohydrate digestibility in *vannamei* shrimp have focussed on the use of cereal flours, including corn flour [5], wheat flour [1,2], and bran flour [6]. However, there is still a lack of information on the use of local carbohydrate sources available in tropical shrimp-farming countries which could be used as raw material for shrimp feed. A study on the availability of potential sources of carbohydrate [8] showed that in the South Sulawesi region there are many resources with potential as feed ingredients. These included, among others; corn flour, sweet potato flour, sago flour, and bran flour. The aim of this study was to evaluate the use of various types of carbohydrate on culture media water quality as well as the proximate composition and glycogen content of *vannamei* shrimp.

2. Materials and Methods

The study was conducted at Universitas Hasanuddin and lasted for two months. Shrimp culture trials took place in the Mini Hatchery, Faculty of Marine Science and Fisheries. Proximate analysis of feed and shrimp and the analysis of shrimp glycogen content were carried out at the Animal Feed Chemistry Laboratory, Faculty of Animal Husbandry. Water quality analysis was conducted at the Water Quality Management Laboratory, Faculty of Marine Science and Fisheries.

2.1. Test animals and culture containers

The test animals used in this study were PL 25 stage juvenile *vannamei* shrimp. The shrimp were sourced from a community hatchery in Maros Regency. The shrimp were adapted to the type of artificial feed used in the trial. The shrimp stocking density was 20 individuals/20 L of water.

The culture containers used in this study were 12 glass aquaria measuring 60cm x 50cm x 50cm with a capacity of 20 L each. The culture medium was sea water diluted to a salinity of 20 ppt.

2.2. Feed formulation and preparation

The study used a low protein feed formulation. The feed ingredients used in the formulations included fish flour, soy flour, shrimp head flour, corn flour, sweet potato flour, wheat flour, bran flour, binders, vitamins and mineral mix. Feed production began with blending all dry ingredients used. The dry feed ingredients were mixed starting with a small amount of raw material and then adding larger amounts and stirring until well blended. The fish oil, vitamins and mineral mix were then added to the dry mixture. Once the ingredients were evenly mixed, warm water was added to form a dough or paste. The feed mixture was stirred until it was no longer sticky when handled. The feed mix was then pelleted using a pelleting machine. The pellet-shaped feed was spread regularly on a tray and dried to dry. The dried feed was stored in labelled plastic bags and kept in a dry place. These procedures were repeated for 4 feed formulations (treatments) using various types of carbohydrate in the feed. These were: Treatment A: corn flour; Treatment B: sweet potato flour; Treatment C: rice bran flour; and Treatment D: wheat flour. The pelleted feed was subjected to proximate analysis in the laboratory to determine the nutritional content of each feed formulation (Table 1).
Table 1. Proximate composition of the feed formulation used in each treatment (mean of five samples)

| Treatment | Water (%) | Crude protein (%) | Crude fat (%) | Crude fibre (%) | NFEa (%) | Ash (%) |
|-----------|-----------|-------------------|---------------|-----------------|----------|--------|
| A         | 6.92      | 26.69             | 12.43         | 6.25            | 34.73    | 12.98  |
| B         | 8.25      | 24.86             | 10.38         | 4.62            | 40.46    | 11.43  |
| C         | 12.06     | 24.39             | 8.49          | 3.73            | 41.05    | 10.29  |
| D         | 11.10     | 25.06             | 9.00          | 3.21            | 41.33    | 10.30  |

aNFE = Nitrogen Free Extract

2.3. Parameter analysis and experimental design
The experimental design used was a completely randomized design (CRD) with four treatments (type of carbohydrate used in the feed) and three replicates per treatment. The treatments were allocated randomly among the experimental units (aquaria). Feeding was carried out with a frequency of four times a day at 10% of shrimp body weight, following [6].

Water quality was sampled at the beginning, middle and end of the study. Water quality parameters measured using an AAS-Spectrophotometer were salinity, turbidity, pH, iron, total phosphorus, ammonia, nitrate, nitrite, H₂S, dissolved oxygen and carbon dioxide. The data were tabulated and mean values were calculated.

Proximate analysis was performed on samples of five shrimp each at the beginning and end of the study. Moisture, crude protein, crude fat shrimp body were measured using the same methods as used for the proximate analysis of the feed, following standard protocols [9]. Moisture content was measured by drying in an oven at 105 °C for 24 hours; crude protein was analysed using the Kjeldahl method; crude fat was analysed through an ether extraction method using the Soxhlet system; and Ash content analysis was carried out by placing the samples in a muffle furnace at 550 °C for 24 hours.

Measurement of shrimp glycogen content was also carried out at the beginning and end of the study following the guidelines in [10]. Shrimp samples were dried in an oven at 70-80 °C. After drying, each sample was finely ground using a pestle and mortar. The resulting powder was wrapped in aluminium foil and placed into a previously labelled plastic bag. This sample was used to analyse shrimp glycogen content using a spectrophotometer.

2.4. Research parameters and data analysis
The study variables comprised water quality, vannamei shrimp proximate composition and vannamei shrimp glycogen content. The data were tabulated and the difference in mean value between treatments for each variable was evaluated using an analysis of variance (ANOVA). Where significant differences were found, this was followed by a post-hoc Tukey test. All statistical analyses were performed using SPSS version 22 software.

3. Results

3.1. Water quality
Water quality parameter data during the study are presented in Table 2. The use of various types of carbohydrate feed did not have a significant effect (P > 0.05) on salinity, turbidity, pH, ammonia, nitrate, H₂S, dissolved oxygen and carbon dioxide. Iron, total phosphorus and nitrite were significantly different (P <0.05) between the treatments using different sources of carbohydrate as feed raw materials. The iron and nitrite concentrations in the culture medium were significantly (P <0.05) higher in units fed with the wheat flour formulation (D) compared to those using corn flour, sweet potatoes and rice bran (A,B,C). The total phosphorus content was significantly (P <0.05) higher in the rice bran treatment (C) than the other three treatments (A,B,D).
Table 2. Water quality parameters during the study

| Parameter          | Treatment | Significance |
|--------------------|-----------|--------------|
| Salinity (ppt)     | A 28,67   | B 29,67      | C 29,67 | D 29,33 | ns |
| Turbidity (NTU)    | 1,15      | 1,00         | 1,53    | 1,54    | ns |
| pH                 | 7,30      | 7,24         | 7,35    | 7,07    | ns |
| Iron (ppm)         | 0,65<sup>a</sup> | 0,68<sup>b</sup> | 0,53<sup>a</sup> | 0,99<sup>b</sup> | * |
| Total phosphorus (ppm) | 3,56<sup>a</sup> | 5,44<sup>b</sup> | 9,74<sup>b</sup> | 5,47<sup>b</sup> | * |
| Ammonia (ppm)      | 0,012     | 0,022        | 0,026   | 0,018   | ns |
| Nitrate (ppm)      | 0,26      | 0,30         | 0,22    | 0,31    | ns |
| Nitrite (ppm)      | 1,26<sup>a</sup> | 1,34<sup>b</sup> | 1,33<sup>a</sup> | 1,68<sup>b</sup> | ** |
| H<sub>2</sub>S (ppm) | 0,0005    | 0,0013       | 0,0000  | 0,0021  | ns |
| Dissolved oxygen (ppm) | 7,15    | 7,47         | 9,17    | 8,43    | ns |
| Carbon dioxide (ppm) | 9,32     | 11,99        | 11,99   | 10,65   | ns |

<sup>a</sup>Different superscript letters on the same line show significantly different treatments: * (P <0.05), ** (P <0.01), ns (not significant)

3.2. Shrimp proximate composition

The results of the proximate analysis on the whole body of the shrimp at the end of the study (Table 3) show that the use of different sources of carbohydrate in the feed produced significantly different (P <0.05) shrimp chemical composition in terms of crude fat, nitrogen free extract (NFE) and ash. However, the differences were not significantly different (P > 0.05) for water content, protein and crude fibre.

Table 3. Whole body chemical composition of vannamei shrimp at the end of the study<sup>a</sup>

| Treatment | Water (%) | Crude protein (%) | Crude fat (%) | Crude fibre (%) | NFE<sup>b</sup> (%) | Ash (%) |
|-----------|-----------|-------------------|---------------|-----------------|----------------------|---------|
| A         | 71,09±1,91<sup>a</sup> | 15,60±0,52<sup>a</sup> | 0,06±0,00<sup>a</sup> | 1,23±0,37<sup>a</sup> | 8,52±1,92<sup>a</sup> | 3,50±0,06<sup>ab</sup> |
| B         | 69,77±3,48<sup>a</sup> | 14,40±0,42<sup>a</sup> | 0,08±0,01<sup>b</sup> | 0,90±0,07<sup>a</sup> | 11,60±2,99<sup>a</sup> | 3,25±0,19<sup>a</sup> |
| C         | 66,79±1,38<sup>a</sup> | 14,66±0,94<sup>b</sup> | 0,07±0,01<sup>c</sup> | 1,34±0,11<sup>b</sup> | 13,47±2,03<sup>ab</sup> | 3,67±0,12<sup>bd</sup> |
| D         | 73,11±3,87<sup>a</sup> | 17,37±0,04<sup>a</sup> | 0,05±0,01<sup>a</sup> | 1,24±0,25<sup>a</sup> | 4,61±3,66<sup>b</sup> | 3,61±0,05<sup>cd</sup> |

<sup>a</sup>Different superscript letters in the same column denote significantly different treatments (P <0.05); <sup>b</sup>NFE = Nitrogen Free Extract

3.3. Shrimp whole body glycogen content

Data on vannamei shrimp whole body glycogen content at the end of the study (Table 4) show that the use of various types of carbohydrate in the feed had a significant effect (P <0.05) on glycogen content.

Table 4. Glycogen content of vannamei shrimp body at the end of the study<sup>a</sup>

| Treatment | Glycogen content (%)<sup>a</sup> |
|-----------|----------------------------------|
| A         | 19,39±0,83<sup>a</sup>          |
| B         | 23,59±0,74<sup>b</sup>          |
| C         | 18,83±0,37<sup>a</sup>          |
| D         | 16,61±0,55<sup>c</sup>          |

<sup>a</sup>Different superscript letters in the same column denote significantly different treatments (P <0.05)
The highest whole body glycogen content obtained from vannamie shrimp fed with the sweet potato feed (treatment B) followed by treatments A (corn flour) and C (rice bran flour), with treatment D (wheat flour) producing the lowest glycogen content. Treatments A and C were not significantly different from each other (P > 0.05), however treatment A was significantly higher and treatment D significantly lower than other treatments.

4. Discussion
During the study period, water quality parameters remained within the range generally considered acceptable for vannamie shrimp culture. It has been suggested that vannamie shrimp are able to tolerate a wide range of salinity including hypo and hyper-saline conditions ranging from 5-50 ppt [11] and even 0.5 to 60 ppt [12]. Thus, the salinity range of 28.67-29.67 ppt in the culture media was well within the appropriate range for growth and survival of vannamie shrimp.

The degree of alkalinity (pH) of the culture medium during the study was in the range of 7.07-7.35. Values suggested for vannamie shrimp culture include an optimum range of pH 7.3-8.5 with a tolerance of pH 6.5-9pH [13] and a more limited range of 7.4-8.9 with an optimum value of 8.0 for intensive shrimp culture [14]. Although slightly below optimum, the pH values recorded during this study are still within acceptable limits for non-intensive shrimp culture.

Ammonia concentrations during the study were in the range of 0.012-0.026 ppm. These values are relatively low, and within the range considered safe for vannamie shrimp. Li and Lovell (1992) in [15] stated that increasing levels of ammonia in culture media is a consequence of high levels of protein in feed. Thus, the toxicity of inorganic nitrogen should be significantly reduced by increasing carbohydrate feed [15]. Furthermore, H₂S levels at low levels of 0.00 - 0.0021 ppm do not affect the survival of cultured vannamie shrimp. The low level of toxic substances in the culture media, including Nitrates and Nitrites produced through the digestion and excretion of proteins used in feed, is likely to be due to the high carbohydrate content of the feed.

Several studies have found that increasing the carbohydrate content of feed can reduce body fat content. During digestive processes, certain monosaccharides can inhibit some amino acids [16]. The presence of glucose can reduce the absorption rate of L-lysine; for example, [17] found that absorption was reduced by 26.64% to 12.34% in the middle intestine and by 23.24% to 5.40% in the posterior intestine at a glucose level of 10 mM [17]. Corn flour has been shown to be more readily used with higher protein deposition in young shrimp than dextrin and glucose [4]. Several researchers have suggested using more complex carbohydrates such as sucrose or starch. Proximate analysis has shown that corn flour has a relatively high starch content, with a reported value of 59.81%; the values for wheat, sweet potato, and bran flours were 45.25%, 47.64% and 22.57%, respectively [8]. In this study, the formulation with corn flour carbohydrate resulted in a slightly higher body protein content of shrimp compared to sweet potato flour, rice bran and flour, however this difference was not significant with relatively large between replicate variation.

Starch can be assimilated after enzymatic hydrolysis. Glucose sourced from starch appears to be absorbed through the intestinal wall at a slower rate than free glucose [4,18]. Negative physiological effects can occur if glucose saturation is caused if the absorption rate in the digestive tract is too high [18]. The use of shrimp feeds formulated with wheat flour, first class clear flour or clear second grade flour produced similar body weight, protein digestibility, dry matter digestibility and identical carbohydrate digestibility in P. monodon [19], indicating that all three types of wheat flour have the same nutritional value.

Previous research indicates that high carbohydrate feed (carbohydrate content above 40%) has potential as a substitute for the commercial feed commonly found on the market for vannamie shrimp culture. This is especially important in the context of current concerns regarding sustainability, and the search for cheap and environmentally friendly fish feeds. High carbohydrate feed is characterized by a high level of digestibility, reducing the residual metabolic waste released into the aquatic environment in the form of nitrogen and phosphorus (N and P). Another concern is the use of fish meal as a source of protein; with decreasing capture fisheries production, the availability of fish meal as a major
component in the manufacture of fish feed will also decrease [20]; optimal utilization of carbohydrates can be one viable alternative to overcome this problem. The ability of vannamei shrimp to utilise protein has been found to decrease as the shrimp grow to larger sizes [21], while [22] has suggested that the feed protein content should be reduced with age, especially for shrimp approaching harvestable size.

An increase in feed carbohydrate content can increase the volume of glycogen stored in the shrimp body; this constitutes and energy reserve which can be used at any time by the animal for metabolism and other activities; for example, using feed formulated with a 40% carbohydrate content significantly increased liver glycogen levels in juvenile GIFT tilapia over a 45 day culture period [23]. Furthermore, liver glycogen content was higher in fish fed glucose than those given wheat flour. In this study, a higher glycogen content was found in the vannamei shrimp given feed formulated with sweet potato carbohydrate (Table 4). This is thought to be related to the high glucose content of sweet potatoes; for example, [18] reports a glucose content of 4.49%, higher than the values found for wheat flour, corn, cassava and sago in the same study.

5. Conclusion
Overall, the results of this study indicate that the use of various feed carbohydrate sources had little impact on the vannamei shrimp culture media. Despite variations between treatments, all parameters measured remained within acceptable limits and water quality was maintained in a condition which should support the growth and survival of vannamei shrimp. The whole body glycogen levels, fat content, NFE and ash content of vannamei shrimp were significantly influenced by the source of carbohydrate used in feed formulation, however, with the exception of glycogen content, the differences were small; the water content, crude protein and crude fibre content were not significantly different. The sweet potato flour feed formulation gave the best overall result with a significantly higher shrimp glycogen content compared to the other three formulations (corn flour, wheat flour and rice bran flour). Further research is recommended on shrimp at different life-stages and at a larger (field trial) scale.

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