

Growth Performance of Milkfish (*Chanoschanos*) Fed Plant-Based Diets

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Abstract: The high cost of fish feeds is the biggest problem in milkfish production caused by the protein from the expensive fish meal. Looking for an alternative from the agricultural commodities is imperative. This paper evaluates the nutritive composition of plant-based formulated feeds utilizing breadnut, breadfruit and banana peelings and their effects on the growth performance (weight gain and length increased) and, the survival rate of milkfish. Results showed that the three formulated feeds have lower protein levels (19.9, 19.6, 20.35) but with extensively higher fat contents (24.4, 15.72, 10.75) than the commercial feed (27.1%, 4%). The samples' growth responses have no significant difference ($p \leq 0.05$) among the four feeds. Thus, the plant-based formulated feeds have the potential to contribute to the growth of milkfish comparable to the commercial feed. Further, the agricultural products used in the formulations are feasible for the replacement of fishmeal and promotion of its utilization in feed production is recommended.

Keywords: Low-cost fish feed, plant protein, formulated feeds, growth characteristics, fish production

1. Introduction

Shortage of fish supply would be between 6,000 – 20,000 tons per year by 2030 (Bell et al.2002; Weeratunge et al. 2011). Thus, milkfish production has to be extensive as it is the most popular of the domesticated variety of hardy and fast-growing fish (Soomro et al. 2015). The high cost of fish feeds is the biggest problem in milkfish production caused by the protein from the expensive fish meal (Adeparusi and Agbede, 2005; Marte et al., 2002). Similarly, Bhosale and his colleagues (2010) declared that in prepared fish diets, fish meal is the most expensive among then ingredients. Reductions in feeding costs can be realized through optimizing nutrient levels of diets, feeding strategies, and by using plant protein sources as fish meal substitutes. Furthermore, practical feeds

need to be formulated using plant protein sources that are locally available (Coloso, 2015).

Although plants or crops may be used to replace fishmeal in fish feed formulation, there are limited studies regarding agricultural products that can adequately replace fishmeal in the formulation. It is, therefore, necessary to determine the effects of plant-based fish feed utilizing agricultural commodities to milkfish production.

Replacement of fish meal with cheaper ingredients from plants reduces production cost (Mahmud et al., 2012).In the Philippines, the utilization of agricultural products as the source of protein in fish feed formulation is insufficient. Soybean meal was the primary plant source to substitute

fishmeal in fish diet, but it is now expensive due to the high demand for this ingredient (Sumagaysay et al., 1991). Hence, using the locally available ingredients that can be incorporated in fish feeds without adverse effects on fish growth is deemed essential since Vuto and his colleagues., (n.d.) declared that feed can be made from locally available ingredients at low cost and compares well with commercial feeds. The need for the development of improving artificial feed for larval milkfish was suggested for further studies (Borlongan et al., 1996). The underutilized and underdeveloped crops such as breadnut (Go et al., 2015; Zamora et al., 2017) and breadfruit (Deivania and Subhash, 2010) are locally available so with the agricultural wastes, banana peelings, and brewers' grain. Nochera and Ragone, (2016) reported the nutritional composition of breadfruit. It contains a low quantity of protein but of excellent quality. Also, it was described as a nutritious, high energy food. Regarding breadnut, Oshodi et al., (2000) confirmed that breadnut flour contained high-quality protein with essential amino acids of 55.1%.

Its protein content is comparable to

soya flour. On the other hand, brewer's grains based on laboratory analysis showed 34.2% of protein. It is a mixture of wheat and corn grains, the waste of brewer's company.

Another waste material is banana peelings used for alternative fishmeal component. However, less work was carried out on determining its nutritional composition (Fas et al., 2015). These made agri-based materials a potential source of feed ingredients.

2. Objectives

This research aimed to formulate plant-based fish feeds utilizing the locally available agricultural products and evaluates their nutritional composition and their effect on the growth performance of milkfish. Specifically, it:

1. Determined the proximate composition of the formulated plant-based feeds used as experimental diets.
2. Investigated the effect of these feeds on water quality, growth performance, and the survival rate of milkfish.

Table 1. Ingredients and cost of formulated feeds (100g)

Treatment 1			Treatment 2			Treatment 3		
Ingredients	(%)	Cost	Ingredients	(%)	Cost	Ingredients	(%)	Cost
Breadnut flour	20	0.8	Breadfruit flour	20	0.4	Banana peelings	20	-
Brewer's Grain	40	0.17	Brewer's Grain	40	0.17	Brewer's Grain	40	0.17
Wheat flour	5	0.22	Bread flour	5	0.22	Bread flour	5	0.22
Soya Meal	5	0.22	Soya Meal	5	0.22	Soya Meal	5	0.22
Water with Concoction	20	0.01	Water with Concoction	20	0.005	Water with Concoction	20	0.01
Fermented soya	5	0.01	Fermented soya	5	0.008	Fermented soya	5	0.01
Vegetable oil	5	0.25	Vegetable oil	5	0.25	Vegetable oil	5	0.25
Total	100	1.67	Total	100	1.673	Total	100	0.87
Cost/Kg		16.7		100	16.73		100	8.73

3. Methodology

3.1 Preparation of materials

Table 1 presents the ingredients of each feed formulation which are all available in the local market. The crops, breadnut (*Artocarpuscamansi*) and breadfruit (*Artocarpusatilis*), were processed to flour using the method described in the studies of Go et al., (2015) and Appiah, et al., (2011) respectively. While the wastes Ingredients: brewer's grain was dried under the sun and powdered, and the banana peelings were boiled and mashed using the meat grinder. They serve as the source of protein and other nutrients. Wheat flour acts as a binder. The wet ingredients include water which was prepared by mixing one liter of water with two tbsp of concoction. A combination of IMO (Indigenous microorganism), FFJ (Fermented Fruit Juice), FPJ (Fermented Plant Juice), LAS (Lactic Acid Serum), FAA (Fish Amino Acid), Calcium Phosphate, OHN (Oriental Herbal Nutrient purchased in the demo farm. It serves as the good bacteria, vitamins, and minerals to avoid using the synthetic ones. Oil, salt, and fermented soya were also used to enhance nutrients content.

3.2 Formulation of Fish Feed

All the dry ingredients (breadnut flour, breadfruit flour, mashed banana peelings and brewer's grain and concentrate) were mixed thoroughly. After that, the wet ingredient was added to the mixture and was blended well then was set aside. Wheat flour paste which serves as the binder was then prepared by mixing the flour to the prepared water and then heated with constant stirring until it turned sticky. The prepared paste was added to the mixture and was mixed well. The mixture was then placed into a meat grinder to form into pellets with a diameter of 2 mm. The pellets underwent the steam

process for 10 minutes later were sun-dried until no traces of water left. The dried feeds were sealed packed with plastic and stored. All formulated feeds have the same procedure.

3.3 Determination of Proximate Composition of Feeds

All samples of formulated feeds and the commercial feed were analyzed in the F.A.S.T. laboratory for the proximate composition as to moisture, crude protein, fat, ash, energy, and sodium. Moisture and ash were determined according to the standard methods of AOAC 1995. Difference calculated the total carbohydrates. For sodium determination, the sample was digested with nitric acid added with potassium chloride solution and diluted to know the volume. The test solution was aspirated through AAS set in flame emission mode for measurement.

3.4 Construction of Cages

The CTU fish pond in Moalboal Cebu was the site of the study. Twelve cages (3 replicates for every treatment) were constructed in the fish pond with the dimensions of 3.0 m x 2.0 m x 1.5 m each. The 12 cages were adjacent to each other and were 12 inches apart and placed horizontally against the current of water. The cages made of bamboo frame and nylon net suspended and weighted to maintain the shape of the flow of water. Four treatments were assigned at random to 12 pens in 3 blocks since this was an experimental study utilizing a Randomized Complete-Block design.

3.5 Experimental fish

Two hundred milkfish fingerlings were purchased from the Oversea hatchery

of CarCar Cebu. Fifty samples were placed in four separate containers and were acclimatized for one week before the experimental samples of the study were selected and placed in their designated cages. The fish samples were trained to eat their assigned feeds (T0, T1, T2, T3) for one week before the study has started. There were 12 cages in all, nine of which for the experimental setup and three for the control set-up. Each cage had 6 m³ of water and contained 12 fingerlings.

3.6 Fish Feeding

Feeding was done based on the study of Adeparusi and Agbede, (2005) wherein the fish samples were fed four times daily at 5% body weight for 90 days. The feeding was done every 6:00 a.m., 10:00 a.m., 2:00 p.m., and 5:00 pm. Every two weeks the weight was measured to adjust the amount of feed. The experimental groups were fed with the formulated feeds (T1, T2, T3) and the control group (T0) with the commercial feed.

3.7 Data collection and analyses on Growth Performances

At the onset of the experiment, the initial length and weight of all fish samples were determined after that data were monitored every two weeks. The experimental cages were checked daily to remove dead fish if any. After 90 days, the samples were harvested, and the final data for length and weight were obtained. Data on growth characteristics such as fish weight gained, length increment, feed conversion ratio, feed efficiency and survival rate were determined using the formulas presented in the study of Amisahet al., (2009) as follows:

- a. Weight Gain (WG)

$$WG = FW - IW$$

where; FW = Final Weight
IW = Initial Weight

- b. Length Increment (LI)

$$LI = FL - IL$$

where; FL = Final Length
IL = Initial Length

- c. Feed Conversion Ratio (FCR)

$$FCR = \frac{TFC}{WGF}$$

where; TFC = Total Feed Consumed (g)
WGF=Weight Gained of Fish (g)

- d. Survival Rate (S)

$$S = (N_0 - N_t) \times 100\%$$

where; N₀= Number of fish sample at the start of the study
N_t= Number of dead fish at the end of the experiment

3.8 Water quality

The water quality was monitored daily to determine the effect of formulated feeds on it. The parameter for water quality such as temperature, dissolved oxygen, and salinity was monitored four times daily (twice both in the morning and afternoon) using a digital DO meter. The pH was determined twice a week. The ammonia and nitrate and nitrite were determined weekly using the API test kit. The criteria for desired water quality were based on the study of Yap, et al., (2007) on Milkfish production and processing technologies in the Philippines. The desired ranges are as follows: DO (3-5 ppm); temperature (22-35 °C); pH (6.5-8.5); salinity (<45 ppt); ammonia (<0.025), nitrate (0.1 – 4.5); and nitrite (< 0.02).

Analysis of Variance (ANOVA) was utilized to determine the significant difference of the formulated feeds on the growth characteristics of milkfish. A Post Hoc test was further employed for significant results for the comparison of means. The computation was done using SPSS version 22.

4. Results and Discussion

4.1 Proximate composition of formulated feeds

Table 2. Proximate Composition of Formulated Feed

Parameters	T0	T1	T2	T3
Crude Protein(%)	^a 27.10	^b 19.90	^b 19.70	^b 20.91
Carbohydrates (%)	43.20	44.16	48.44	46.07
Crude Fat (%)	^a 6.00	^b 24.4	^c 15.72	^d 10.75
Fiber(%)	9.00	5.91	3.85	8.11
Ash (%)	14.50 ^a	^b 2.74	^b 2.48	^b 3.80
Moisture (%)	10.20	8.80	10.54	10.61
Sodium, ppm	^a 0.19	^b 430.30	^c 250.30	^d 363.2

Legend: Same superscripts do not significantly ($p \leq 0.05$) differ

The results of the proximate compositions of the formulated and control feeds are presented in Table 2.

Protein. Proteins are compounds formed through linkages of amino acids. There are over 200 naturally available amino acids, with 20 of these compounds commonly consumed by various species (Craig, et al., 2017).

However, of the 20 amino acids, 10 of these are not synthesized by the fishes, including methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine and phenylalanine(Chafman & Miles, 2006). Of the following amino acids, lysine and methionine are often considered as the first limiting amino acids (Craig, et al., 2017). Typically, protein is utilized for fish growth when adequate proportions of fats and carbohydrates are made available in the diet if not, protein can still be used as energy source and life support rather than growth Craig, et al., 2017).

The crude protein contents (Table 2) of the formulated feeds showed that commercial feed contained the highest protein followed by T4 (banana peels) while T1 and T2 had almost the same amount. Results further revealed that there were no significant ($p < 0.01$) differences in the protein contents of the three formulated feeds. However, when compared to the control feed, their protein contents were significantly ($p < 0.01$) lower.

Protein inclusion is the most expensive part of a fish feed; thus it is essential to meet and match the adequate and minimum protein requirement and amino acid requirement of each cultured fish species (Banrie, 2013). A fish’s protein requirement is based on several factors including water temperature and water quality as well as its genetic composition and feeding rates (Craig et al, 2017). Providing high levels of protein in an animal diet is both economically and environmentally unreasonable, since as indicated by Banrie (2013) protein components are one of the very expensive dietary constituents, and the inclusion of excessive proteins increases the fish’s nitrogenous waste excretion as well.

Carbohydrates. In most animal diets, carbohydrates are considered as one of the primary sources of energy. These macromolecules are usually classified based on their sugar components, structural form, composition, the degree of polymerization as well as glycosidic bondages. Samples of carbohydrates include oligosaccharides (i.e., lactose, maltose), polysaccharides (i.e., starch, chitin, cellulose) and monosaccharides (i.e., glucose, fructose) (Abro, 2014). The determination of the nutritional effects of carbohydrates usually relies on their individual properties, including digestion and absorption rate, viscosity, as well as water-binding capacity and fermentation ability in the gastrointestinal tract (Abro, 2014).

Regarding the carbohydrates compositions of the experimental and control feed, the highest value was observed in T3 (breadfruit) and the least value was in the control feed. However, the carbohydrates content among the four feeds were comparable as an insignificant result was obtained using the test of Analysis of Variance (ANOVA).

The appropriate amount of carbohydrates should be provided in fish feeds so to ensure the best results on nutrient utilization, growth, metabolism, and health of fishes (Abro, 2014).

Milkfishes as warm water fishes are known to utilize carbohydrate more efficiently than cold water fishes. Also, as an omnivorous species which feeds on the lower trophic level, like the tilapia and carp, milkfishes can efficiently utilize high dietary levels of carbohydrates at about 30-50% as compared to higher trophic level carnivorous fishes (Enes et al., 2011). However, no particular dietary carbohydrate amount has been defined for fish growth.

Crude Fat. Fats are high-energy nutrients that can be utilized as a protein substitute in aquaculture feeds (Craig, et al., 2017). In fact, lipids supply about twice the energy of proteins and carbohydrates; and these compounds typically comprise about 15% of fish diets, supply essential fatty acids (EFA) and serve as transporters for fat-soluble vitamins (ibid).

The control and the formulated feeds have different amounts (Table 2) of crude fat. Among the formulated feeds, Treatment1 (breadnut) contained significantly higher amount (24.4) followed by T2 (breadfruit) then T3 (10.75). The fat compositions of these feed were significantly higher than the control feed as it had the least fat content (6.0). The high-fat contents of the formulated feeds compensate for their low protein compared to the control feed as the recent trend in fish feeds is to use higher levels of lipids in the diet. Although increasing dietary lipids can help reduce the high costs of diets by partially sparing protein in the feed, problems such as excessive fat deposition in the liver can decrease the health and market quality of fish.

The critical components of feeds are protein, fat, and carbohydrates (Cheng et al., 2005; Craig and Helfrich, 2002). The role of protein in fish feed is for fish growth provided that both fat and carbohydrates are sufficient; otherwise, protein will be used as a source of energy (Cheng et al., 2005 and Craig and Helfrich, 2002). Hence, it can be implied that fat, high energy nutrients, can be utilized as a substitute for protein in feed formulation (Craig and Helfrich 2002). Thus, incorporating high fat in fish diets is the latest trend in feed formulation.

Fiber. As described by Davies, (1985) fiber refers to the present material

which is neither digested nor absorbed by the animal, which provides the physical bulk or substance to a ration.

The fiber content among the experimental treatments revealed that T3 (banana peel) obtained the highest percentage. This result was supported by the experiment conducted by Shankar et al. 2017 that banana peels are rich in dietary fiber. The least amount was observed in T2 (breadfruit). However, when compared to the control feed, the control feed had the highest fiber content of 9.0 than the experimental feeds. However, their fiber compositions did not significantly differ. As indicated by De Silva and Anderson, (1994) it is not recommended for fish feeds to contain fiber contents beyond 8-12% since these amounts would result in the decrease in quality of unusable nutrient. Thus, the fiber contents of these feed were within the acceptable range.

The variation of crude fiber content in several feedstuffs poses significant effects on the animal's ability to absorb and digest dietary fiber. As a monogastric species, fishes have an intestinal microflora which is not capable of breaking down dietary fiber since these animals do not possess endogenous enzymes that can catalyze the degradation of cellulose and other fibrous components within the diet (Stickney & Shumway, 1974). Still, the inclusion of dietary fiber in fish feeds is only beneficial to fishes in amounts not exceeding the fishes' maximum tolerance. Any value provided beyond the fishes' maximum tolerance on dietary fiber would provide adverse effects on the growth performance of the animal as this lowers the digestibility of nutrients (De Silva and Anderson, 1994; Li et al., 2012).

When appropriate amounts of crude fiber are included in the fish feed, various health benefits can be attributed which can be manifested in the animal's length and size growth. In fact, crude fiber plays a significant role in fish health by effectively removing toxins and waste products due to its ability to bind to water thus aiding the animal in producing firmer stools as well (Cai-Juan, et al., 2016). Moderate inclusions of fiber in feeds permits better binding as well as feed passage within the alimentary canal.

Ash. The ash percentage in feed diets represents the mineral content within the feeds as a whole, including minerals such as calcium, phosphorus, potassium, and magnesium.

Regarding the ash contents of the four treatments, the control feed obtained significantly higher amount (14.5) compared to the three formulated feeds (T1, T2, T3) which have ash content ranging from 2.48-3.78 only. These values are less than the required ash content in feeds since according to Chafman & Miles (2015) the standard ash content in fish feeds usually ranges from 7% to 12%. The result implies that formulated feeds contain fewer minerals compared to the control feed. Thus, they need to be supplemented with minerals to provide better growth performance to fish. On the other hand, the obtained ash of the control feed exceeds the required limit. Thus, it may have detrimental effects on the fishes, including increased mortality and reduced growth (Shearer et al., 1992; Chafman & Miles, 2006).

Moisture. Feedstuff with higher moisture content is suitable for milkfishes which live in the oceans and salty water habitats. High moisture-containing feedstuff, when left in salty water, does not become

wet and dissolve quickly, as compared to fish feeds with lower moisture (Cai Juan et al., 2016). Low moisture content in feedstuff are those containing only 6.5% to 5% moisture; in comparison with the experiment feedstuff, all the treatments in the study are identified with high moisture content. At high moisture content, pellet breakage is less, thus feed spill is usually avoided as compared to feedstuff with low water content (Aas et al., 2011). Avoiding feed spillage caused by small particles and fine substances would maximize feed intake and feed utilization in fishes (Oehme et al., 2012).

Sodium. It is one of the essential elements needed by fishes to ensure a regular physiologic activity; this mineral influences the animal’s reasonable regulation of osmotic pressure keeps digestive processes normal by ensuring the production of acid in the stomach.

The sodium composition of the formulated feeds was measured in ppm. The obtained sodium of the four treatments is reflected in Table 2. The data show that the sodium contents of the four feeds were significantly different. T1 (breadnut) has significantly higher content followed by T3 and T2; The control feed obtained the least amount.

The addition of sodium in the form of salt makes the food more palatable and has several benefits to fish diets such as boosting the fish’s appetite and acting as humectants through reducing water activity. The inclusion of salt in the fish feeds yields significant results on fish growth and performance (Towers, 2018).

4.2 Growth Performance

Table 3 presents the growth performance and survival rate of milkfish

Table 3. Growth performance and survival rate of milkfish fingerlings for 90 days

Parameters	T ₀	T ₁	T ₂	T ₃
Ave. initial weight (g) (AIW)	32.40	37.60	31.80	36.53
Ave. initial length (cm) (AIL)	14.90	14.50	14.20	14.30
Average final weight (g) (AFW)	169.70	167.70	160.20	168.80
Ave. final length (g) (AFL)	21.30	21.46	21.20	21.30
Ave. weight gain (g) (AWG)	137.30	130.10	128.40	132.22
Ave. length increment (g) (ALI)	6.44	6.96	6.99	6.97
Feed Conversion Ratio (FCR)	2.63	2.90	3.62	3.25
Survival rate (%) (SR)	100.00	100.00	100.00	100.00
Feed efficiency (%) (FE)	38.02	34.48	27.60	30.80

fingerlings (*Chanos Chanos*), fed on four different feeds for 90 days. Results revealed that among the formulated feeds, T3 (banana) showed better growth performance for it obtained the highest WG next to the control feed and the lowest value was seen in the samples fed with T2 (breadfruit). This result may be due to the slightly higher content of protein in T3, but its fat content was the lowest compared to the other two treatments (T1 and T3). However, ANOVA Analysis showed no significant (p<0.01) difference in the WG and LI of the four treatments. Though the highest average WG was observed on control feed, however, the value was comparable with the other treatments. It means that the effects of the three formulated feeds (T1, T2, T3) on the

growth of fish samples is comparable to the effect of the control feed.

The obtained results in the study were contrary to the statement of Magondu et al. (2016) as they said that feeding the fish on formulated diets resulted in significantly higher mean fish weight gain than that of fish fed on non-formulated diets because the highest weight gain was observed in the fish samples fed on commercial feed.

Control feed has the lowest value of FCR and highest FE followed by T1 while T2 obtained the highest value of FCR. It means that a lesser amount of T1 (breadnut) feed is required to get a kilo of fish compared to T2 and T3. Conversely, T2 does not only show poor performance in fish growth, but it also requires the highest amount of feed to provide a kilo of fish compared to the other two treatments (T1 and T3).

The FCR values obtained in this study were lower than the FCR values obtained by Mwangamilo, and Jiddawi,(2003) on milkfish fed diets with different levels of protein. It may be due to the higher protein levels they utilized than in this study. Moreover, the FCR and FE values obtained in this study were not in the range of “good growth” since according to Craig,(2002), the feed of good growth should have an FCR value of 1.5-2.0 or FE value of 50%. It indicates that the formulated feeds need to be improved to ensure optimal growth of milkfish.

4.3 Survival Rate

All fish samples have survived for the entire period of the study (see Table 3). Thus, the formulated feeds have no harmful effects on the fish samples. A similar result was observed with that of Mwangamilo and Jiddawi (2003).

4.4 Water Quality

The water quality parameters observed in the study for 90 days are reflected in Table 4. Results showed no significant ($p < 0.05$) difference in all the parameters of the five treatments. Moreover, all values are within the range of standard values. It showed good water quality for the whole period of study. It implies that the formulated feeds had no adverse effect on the water quality that may affect the health and survival of fish.

Moreover, the excellent water quality maintained over the entire period was due to the speed of current generated by winds which produce waves that resulted to good water exchange in all cages. As a result, no uneaten food stocked on the cage and the build-up of ammonia and nitrates were prevented.

Table 4. Effect of treatments on water quality parameters

Parameter	To	T1	T2	T3
Dissolved Oxygen (mg/L)	4.6	4.6	4.5	4.7
Temperature (°C)	28.6	28.6	28.7	28.7
pH	7.4	7.4	7.4	7.4
Ammonia	-	-	-	-
Nitrate	-	-	-	-

(-) means not detected

5. Conclusion and Recommendation

The plant-based formulated feeds have the potential to contribute to the growth of milkfish comparable to the commercial feed. They are as well feasible to replace fishmeal in feed formulation to reduce the price of feed. Thus, agricultural products like breadnut, breadfruit, brewer's grain and especially banana peels should be

promoted for their utilization in feed formulation.

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