Apparent Nutrient Digestibility of Plant Based Diets by *Tilapia rendalli* (Boulenger, 1896)

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Abstract

This study investigated the digestibility of diets formulated exclusively out of plant sources fed to *Tilapia rendalli*. The study was conducted at NkhataBay Fisheries Laboratory along Lake Malawi for 21 days. The experiment was laid out in a Completely Randomized Design (CRD) using 30x30x35cm glass aquaria with each of the four experimental isonitrogenous diets containing varying plant sources replicated three times. Juvenile *Tilapia rendalli* (25.0 ± 1.0 g) were conditioned for 5 days to accept the artificial dry plant feed fed two times a day. Faeces were collected by stripping method using a tube and pipette, preserved in beakers and later analyzed for chemical composition. An indirect method of measuring digestibility was used to calculate the apparent digestibility coefficients (ADCs) of the diets containing 1% Chromic oxide and faeces. Water quality data was measured on daily basis using a spectrophotometer. Data was analyzed using Analysis of variance (ANOVA) at P=0.05 using R- statistical software. Protein digestibility coefficient ranged from 30.82% ± 0.81 to 29.21% ± 0.91. However, apparent digestibility coefficients for gross energy were slightly higher than those of other elements. Results show that nutritional and digestibility value provide a good support for the development of a system of selecting ingredients for inclusion in fish diets.

Keywords: *Tilapia rendalli*; Plant diets; Digestibility; Faeces; Gross energy; Crude protein

Introduction

Tilapia has been identified as one of the species with greatest potential to contribute to fish production in Malawi. Previously, Tilapia was consumed mainly in Africa and Asia but nowadays it has been touted as the “new white fish” replacing the depleted ocean stocks, leading to a worldwide demand for the fish. Globally, fish feeds usually contain high levels of dietary protein, supplied especially by fish meal, the main protein source for aqua feed Deng et al. Wu et al. Glencross et al. [1,2]. However, there are many risks associated with availability, price and quality fluctuations of fish meal in the international market. Therefore, In order to enhance aquaculture production, improve food security and reduce the level of poverty in developing countries, a search for cheap and locally available feedstuffs is required Munguti et al.

In Malawi, *Tilapia rendalli* can play an important role under extensive/semi-intensive fish pond culture system as it feeds mainly on readily available macrophytes. *Tilapia rendalli* is a promising alternative candidate because of its ability to utilize plant protein which is relatively cheap compared to the conventional fishmeal. According to El-Sayed, *Tilapia rendalli* exhibits desirable attributes including feeding at low trophic levels (feed largely conditions such as temperature, low dissolved oxygen levels, high ammonia levels, it has a fairly fast growth rate and ability to reproduce readily in captivity and does not incubate eggs in the mouth.

Sustainability of aquaculture will depend on the replacement of fish meal by alternative protein sources in aqua feeds [2-4]. However, finding alternative plant protein sources in aquaculture feeds is a challenging task. Plant proteins have limitations that impede its potential. Fibres are indigestible and nutritionally unavailable carbohydrates in animal and plant sources and are usually used as fillers and binders in fish feeds. Fish can tolerate up to a certain amount of dietary fiber which is generally >10% before it start affect growth rate. Presence of Antinutritional factors that are deleterious to health of fish and also low amino acid and protein are other potential hazards of plant proteins.

The nutritional value of a given feedstuff is based not only in its chemical composition, but also in its nutrients and energy availability. Availability of nutrients for fish should be defined mainly items of their digestibility, that is, the fraction of nutrients from the ingested feed and feed ingredients that will not be excreted in the faeces. Digestibility determination is one of the nutritional assessment tools that must be employed to deduce the availability of nutrients levels in the plant diets that can be turned into fresh. Determination of digestibility is the first step in evaluating the potential of an ingredient for use in the diet of an aquaculture species [1]. Nutritive value of feeds is determined by a number of factors, including composition, odor, texture and taste. The apparent digestibility of nutrients like protein, energy and individual amino acids are of prime consideration as the basis for feed formulation and for experimental purposes in fish in the path of results of Portz and Cyrino [4], this study aims at evaluating the digestibility of exclusively formulated plant protein based diets by a model herbivorous fish, the *Tilapia rendalli*. The information gained will be utilized in the feed manufacturing industry to foster the use of alternative, plant proteins in diets for intensive production of Tilapia.

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Materials and Methods

Collection of locally available plant ingredients

An observational survey by farmers and researchers was conducted in Mpamba; Nhkathay northern Malawi to identify locally available plants for assessment of their nutritional profile. The aim was to ascertain the plant’s potential for inclusion into diets for pond raised fish (tilapias species). Selection of the feedstuffs was based on both temporal and spatial availability, abundance; competition for other uses, prior nutritional profile from literature and cost implication. The following plant ingredients were collected: Cassava (Manihot esculenta) peels and leaves, Pawpaw (Carica papaya) leaves, Sweet potato (Ipomea batatus) leaves, meal and tubers, Jackfruit (Artocarpus heterophyllus), Mexican fireplant (Euphorbia heterophylla), Black jack (Bidens pilosa), Cocoyam (Colocasia esculenta) leaves, Banana (Musa balbisiana) leaves, Maize (Zea mais) bran, and Ake (Blighia sapid) leaves.

Processing of plant ingredients

All plant ingredients were dried in the sun for three days to reduce their toxicity [5]. Drying of ingredients was done not only to reduce toxicity but also to mill them into powder form ready for proximate analysis. Ingredients were milled using a mortar and pestle. Finally, the ingredients were then sieved using a wooden sieve to remove cellulose and lignin materials to remain with the powder.

Proximate analysis of plant feedstuffs

Proximate analysis of the plant ingredients was done at Department of Aquaculture and Fisheries Science laboratory Lilongwe University of Agriculture and Natural Resources (LUANAR), Bunda College Campus, The plant samples were analyzed for crude protein, crude fiber, crude fat, ash and moisture content done in triplicate, following the procedure outlined by the Association of Official Analytical Chemists AOAC [6].

Fish and experimental condition

This study was conducted at the Department of Fisheries Science Laboratory, Mzuzu University located in NkhataBay, northern Malawi. Each treatment had three replicates, 10 Tilapia rendalli per replicate, with mean initial fish weights of 25 ± 2 g. Each treatment was randomly assigned to three glass aquaria (measurement in L). Which replicate, with mean initial fish weights of 25 ± 2 g. Each treatment was replicated twice were pooled and kept in beakers to dry until analysis. Prior to the analysis, samples from the rest of the days from fish on each experimental diet were pooled together and analyzed.

Feed ingredient and diet preparation

Locally available plant ingredients obtained from Mpamba Nhkathay were evaluated for proximate composition (Table 1). The ingredients were Cassava leaf and flour, sweet potato leaf and meal, Black jack and Cocoyam leaf. The fresh plant sources were dried in the sun for three days to remove anti-nutritional factors as recommended by Francis et al. [3] before being milled using a traditional mortar and pestle. The experimental diets were formulated using the trial and error method. Chromic oxide (Cr₂O₃) was used as an inert marker in reference diet (Table 2). Each test ingredient was ground and sieved to produce powder. Diets were mechanically mixed with warm water to make dough which was later used to produce pellets. The resultant moist pellets were then dried at under a shade for approximately 12 h. After that, the feeds were then broken up and sieved into convenient pellet sizes (2-3 mm) and were stored in polythene bags at 4°C. The amount of diet needed weekly was then kept at room temperature [7].

Experimental procedure

During acclimatization period, fish were fed with experimental diets for 7 days prior to the beginning of fecal collection. All fish were fed two times at 09:00am and 2:00pm respectively at the rate of 4% of their body weight on a daily basis during 21 days. On termination of the experiment, fish were weighed individually and mean weight for each aquarium was calculated. One hour after the feed was administered; any feed and feces present in the aquaria were removed.

Feacal collection

Faecal matter was collected from the aquaria by using a siphon and a pipette into a petri dish. Faecal collection was done within 2 hours of voiding during the day and the fecal material voided during the night was collected next morning at 07:00 hours. Faecal collection was done for 21 days. Samples from two replicates in each treatment replicated twice were pooled and kept in beakers to dry until analysis. Prior to the analysis, samples from the rest of the days from fish on each experimental diet were pooled together and analyzed.

Biochemical analysis of fecal and feed material

The fecal and feed material for the entire experimental period were pooled in triplicates samples and then analyzed for Crude protein, Ether extract (fat), fiber, ash, following the procedures stipulated by the Association of Official Analytical Chemists [6] while gross energy was analyzed using a bomb calorimeter and chromic oxide was determined according to Fenton & Fenton. Total nitrogen (N) was determined by the Kjeldahl method and CP content was calculated as N × 6.25. Ether extract was determined by Soxhlet extraction without acid hydrolysis. Ash was the residue after ashing the samples at 550-600°C. Fiber content determined using acid-base digestion according to Robertson and Van Soest.

Ddigestibility determination

Apparent digestibility coefficients (ADCs) of each nutrient in the

Table 1: Percentage ingredient composition of the experimental diets fed to Tilapia rendalli.
Values (Mean ± SE) in a column with different superscript letters are significantly different (P<0.05).

Table 2: Proximate composition of plant ingredients from Mpamba (Mean ± SE) expressed as percent (%) dry matter.

| Element Analyzed | Crude protein | Energy Kg/j | Fat | Fibre | Ash | Moisture (DM) |
|------------------|--------------|-------------|-----|-------|-----|---------------|
| Treatment 1      | 61.88 ± 0.25a | 15.25 ± 0.05a | 24.04 ± 0.14a | 0.32 ± 0.006a | 0.33 ± 0.03a | 36.78 ± 0.03a |
| Treatment 2      | 63.21 ± 0.03a | 14.69 ± 0.02a | 22.69 ± 0.10a | 0.37 ± 0.006a | 0.35 ± 0.08a | 34.91 ± 0.03a |
| Treatment 3      | 62.71 ± 0.07a | 15.78 ± 0.06a | 27.2 ± 0.28a  | 0.38 ± 0.006a | 0.34 ± 0.03a | 34.76 ± 0.03a |
| Treatment 4      | 62.38 ± 0.03a | 15.36 ± 0.04a | 26.64 ± 0.08a | 0.36 ± 0.003e | 0.34 ± 0.03a | 32.43 ± 0.03a |

Values (Mean ± SE) in a column with different superscript letters are significantly different (P<0.05).

Table 3: Proximate composition (%) of the whole body carcass of *Tilapia rendalli* fed on pelleted diets containing different plant feedstuffs.

| Element Analyzed | Crude protein | Energy Kg/j | Fat | Fibre | Ash | Moisture (DM) |
|------------------|--------------|-------------|-----|-------|-----|---------------|
| Diet 1           | 29.52 ± 0.07a | 10.99 ± 0.09a | 9.57 ± 0.12a | 4.28 ± 0.04a | 14.76 ± 0.05a | 92.52 ± 0.06a |
| Diet 2           | 30.5 ± 0.05a | 10.81 ± 0.09a | 10.95 ± 0.05a | 4.31 ± 0.09a | 14.3 ± 0.05a | 93.14 ± 0.02a |
| Diet 3           | 29.31 ± 0.19a | 11.18 ± 0.02a | 11.5 ± 0.06a | 4.02 ± 0.07a | 14.1 ± 0.03a | 92.64 ± 0.04a |
| Diet 4           | 30.82 ± 0.81a | 10.38 ± 0.02a | 9.5 ± 0.11a  | 4.49 ± 0.02a | 15.06 ± 0.01a | 93.55 ± 0.34a |

Values (Mean ± SE) in a column with different superscript letters are significantly different (P<0.05).

Table 4: Proximate composition (%) of feed containing different plant feedstuffs fed to *Tilapia rendalli*.

| Element Analyzed | Crude protein | Energy Kg/j | Fat | Fibre | Ash |
|------------------|--------------|-------------|-----|-------|-----|
| Diet 1           | 30.44 ± 0.29a | 43.44 ± 0.59a | 67.78 ± 1.35a | 23.77 ± 0.95a | 3.82 ± 0.66a |
| Diet 2           | 31.45 ± 0.34a | 31.50 ± 0.36a | 61.54 ± 0.91a | 39.57 ± 0.84a | 4.34 ± 1.35a |
| Diet 3           | 30.04 ± 0.06a | 31.04 ± 0.34a | 61.65 ± 0.71a | 26.93 ± 0.76a | 4.82 ± 0.72a |
| Diet 4           | 24.15 ± 0.28a | 21.56 ± 0.34a | 54.29 ± 2.22a | 24.29 ± 0.59a | 11.80 ± 1.61a |

Values (Mean ± SE) in a column with different superscript letters are significantly different (P<0.05).

Table 5: Apparent digestibility coefficients (%) of plant diet fed to *Tilapia rendalli* (DM).

| Results |
|----------|
| **Apparent digestibility coefficients (ADCs)** |
| The apparent digestibility coefficients determined by indirect method of digestibility as reported in Table 3. |

| Protein digestibility |
|-----------------------|
| The protein digestibility coefficient ranged from 31.45% to 24.15%. |

| Fat digestibility |
|-------------------|
| The fat digestibility coefficient ranged from 67.78% to 54.29%. |

Data analysis

Apparent digestibility coefficients for protein, fat, fiber, ash and energy were subjected to a one-way analysis of variance within each collection method using the R statistical software. Diet was considered the fixed effect, whereas replicate tank within diet was considered the random effect. Differences in ADCs among diets were determined according to Duncan’s multiple range test mean comparison Duncan, [8] and were considered significant at P < 0.05.
Ash digestibility

The fat digestibility coefficient ranged from 11.80% to 3.82%. There were significant differences among the digestibility of ash in different diets P (<0.05). But ash digestibility for diets 1 and 2 was not significantly different P (>0.05) However, diet 4 (11.80%) had the highest digestibility coefficient, followed by diet 3 (4.82%), then diet 2 (4.34%) and lastly diet 4 (3.82%) (Table 5).

Crude fibre digestibility

The fat digestibility coefficient ranged from 39.57% to 23.77%. There were significant differences among the digestibility of fat in different diets P (<0.05). But fibre digestibility between diets 1 and 4 was not significantly different P (>0.05). However, diet 2 (39.57%) had the highest digestibility coefficient, followed by diet 3 (26.93%), then diet 4 (24.29%) and lastly diet 1 (23.77%) (Table 5).

Gross energy digestibility

The gross energy digestibility coefficient ranged from 43.44% to 21.56%. There were significant differences among the digestibility of energy in different diets P (<0.05). But gross energy digestibility between diets 2 and 3 was not significantly different P (>0.05) However, diet 1 (43.44%) had the highest digestibility coefficient, followed by diet 2 (31.50%), then diet 3 (30.04%) and lastly diet 4 (21.56%) (Table 5).

Discussion

The average values of faeces composition and the diets digestibility coefficients for Tilapia rendalli are presented in Table 5. The treatment 1 presented the highest values (ADCGE) of for energy (ADCGE) and are presented in Table 5. The treatment 1 (31.50%), then diet 3 (30.04%) and lastly diet 4 (21.56%) (Table 5). There were significant differences among the digestibility coefficients, followed by diet 3 (4.82%), then diet 2 (4.34%) and lastly diet 4 (3.82%) (Table 5).

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Fish use around 80% of dietary dry matter (ADC DM ) which describes how efficiently the feeds or feed ingredients are digested, and how much of their nutrient contents can be made available to fish for maintenance and growth. In addition, (ADC DM ) generally provides a better estimate of the quantity of indigestible materials in the feeds or feed ingredients, rather than that of the individual nutrient.

Nutritional values of proteins and protein sources vary as a function of amino acids profile and digestibility. The proportion (%) of crude protein, CP (i.e., Nitrogen) in a feed ingredient can either originate from protein or nonprotein nitrogen. Therefore, ingredients with high CP from non-protein nitrogen will not contribute adequate amino acids in tandem with the nutritional requirements of fish. Thus, such ingredients will on the other hand, lead to increased production ammonia and other nitrogenous wastes by the fish thereby lowering productivity and water quality of production systems [9-11].

Digestibility coefficients in this study were generally in contrast and generally lower compared with other workers on plant diets. The protein digestibility values for protein-rich feed ingredients are usually in the range of 75–95%. For example, protein digestibility values sunflower cakes 86–89% and wheat bran 75% [12]. This is in agreement with the findings of Fontainhas-Fernandes et al. [13] who reported defatted soybean meal 94.4%, full-fat soybean meal 90% and micronized wheat 88.6%. In the same vein, Mhabinizire et al. [14] reported values that ranged from 70 to 89% in tilapia for Cottonseed meal and corn gluten meal 89% [11], corn meal 83-84% [15] and Cottonseed meal 81.8% [16] for Nile tilapia, Oreochromis niloticus.

Water quality parameters have a profound effect on the performance of fish in aquaculture and may affect feed intake as well as digestion. In the present study, water temperature records ranged from 21-22°C which is absolutely low as the optimum temperature range for Tilapia is 20-30°C with better results obtained at between 26-30°C [9]. Thus, the temperatures recorded may have contributed to low protein digestibility as the metabolic demand was also low.

Earlier studies have shown that Tilapias have the capacity to utilize a large number of alternative plant and animal protein sources [17]. Tilapia has a digestive tract that is relatively long, as in herbivorous fish, and shows morphological and physiological adaptations for the utilization of diets high in fibre [12,18]. This is consistent with previous work that has shown that Tilapias are capable of digesting and absorbing relative large amounts of carbohydrates [18]. Although Tilapias have an adaptation to digest plants diet, composition of nutrients of the diet influences digestibility. The source of the ingredients making up a diet can either be animal or plant based and has an effect on digestibility due to varying nutrient make up. Most plant based sources have high fibre contents especially the leafy parts unlike the animal sources. In the present study, the diets were exclusively made from plant sources and these may be responsible for low protein digestibility coefficients. Dietary plant ingredients can affect gastrointestinal transit time of feed as a result of the presence of fibres and sugars, and alter the digestibility of nutrients ingested by the fish (Storebakken et al.) [2,19]. According to Eusebio et al. [19], as dietary fibre is part of the carbohydrate component of plant ingredients, most fish cannot utilize it. However, low dietary concentrations of dietary fibre (3-5%) may have a beneficial effect on fish growth. On the other hand, high dietary fibre (>8%), may decrease dry matter digestibility of the diet and reduce the availability of other nutrients [20]. High dietary carbohydrate contents reduce the activity of proteolytic enzyme in fish. In the present study the fibre digestibility coefficient ranged from 23.7%-39.57% and therefore liable enough to impede digestibility of the diet and lock the bioavailability of other nutrients like protein.

Dietary energy is the second most important factor affecting the utilization of feeds by fish after protein. Fish are known to feed to satisfy their energy requirements, and if the diet does not contain sufficient energy levels, protein is used for energy rather than for growth [21]. Protein is the most expensive component in fish feeds and plays an important role in growth of fish (NRC, 1993) [22]. Previous studies demonstrated that providing properly balanced ratios of protein to non-protein energy in diets can spare dietary protein from energy metabolism and then increase its utilization for fish growth diets [23-28].

In the current study, the Apparent Digestibility Coefficient of gross energy (ADCE) range from 43.44% to 21.56% slightly higher than the Apparent Digestibility Coefficient (ADCE) range for crude protein (31.45%-24.15%). An inadequate dietary protein dietary protein to energy ratio may result in lower growth as well as low protein and energy utilization in fish [28]. In diets with low protein to energy ratio, the use of dietary protein for growth and maintenance of body protein is maximized, while in diets with high protein to energy ratio, more protein is used for energy or stored as fat [28]. According to (Van der Meer et al., 1997), the protein sparing effect of energy occurs only if the
minimum protein requirements are met, including adequate amounts of amino acids. In addition, Apparently Digestibility Coefficient of gross energy (ADC_{gr}) range from 43.44% to 21.56% slightly lower than the Apparent Digestibility Coefficient for crude fat (ADC_{cr}) range for crude protein (67.78%-54.29%). Providing adequate energy from dietary lipid can minimize the use of protein as an energy source [29]. Therefore, the presented study infers the protein sparing effect of plant diets as evidenced from the digestibility coefficients.

From the foregoing discussion, it can also be suggested that the digestibility coefficients from almost all diets analysed in the present study were lower than those reported in other studies on Tilapia due to poor utilization of the diets owing to several reasons, presence of anti-nutritional the that impeded feed intake and subsequently digestion due to off flavors and toxicity nature although not investigated in the present study. These results agree favorably with Mbahinireki et al. [14] who reported in his investigation of Cottonseed-meal (CSM) as a major source of protein for Tilapia (Oreochromis species). Results indicated presence of up to 0.11% to 0.44 % gossypol and that was identified as a major limiting factor for acceptance of the CSM diets in tilapia farming.

**Conclusion**

The potential of feedstuffs such as leaf meal in fish diets can be evaluated on the basis of its proximate chemical composition, comprising the moisture content, crude protein, crude fiber, crude lipid, total ash and nitrogen-free extract. Results of the present study show that the crude protein content of Cocoyam, Cassava leaf and Black jack were higher than all ingredients analyzed. Digestibility coefficients indicate diets exclusively formulated from plant sources tested on Tilapia rendalli that have a low digestibility potential and subsequently less nutrient available for growth and energy. Therefore, knowledge of the digestibility of protein helps the farmer to deduce whether or not feed is meeting the targeted requirements of fish.

**Recommendation**

Based on proximate composition of the ingredients and digestibility coefficients of different diets fed to Tilapia rendalli, the study suggests inclusion of plant feedstuffs in Tilapia diets in order to minimize the cost of fish production among small scale fish farmers. The plant feedstuffs must however, be rid of anti-nutrition factors which negate growth of fish. To this end, application of heat through boiling, drying or roasting could be some of the methods that need to be advocated to rural fish farmers across the country to be performed before using the ingredients. There is also need for further research to evaluate among others, the amino acid profile, and anti-nutritional factors of the plant feedstuffs and as well as the diets as these have a profound effect on performance fish.

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