International Scholars Journals

African Journal of Fisheries Science ISSN 2375-0715 Vol. 7 (1), pp. 001-006, January, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Digestibility coefficients of processed jackbean meal *Cannavalia ensiformis* (L.) DC for Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) diets

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Accepted 5 October, 2018

A study was conducted to determine *in-vivo* apparent digestibility coefficient of nutrients in processed jackbean meal. Acid Insoluble Ash (AIA) was used as an indicator. The test diets consist of the seed meal replacing soybean meal at 10, 20, 30 and 40% to serve as test diets D10, D20, D30, and D40 respectively. A diet called as diet CTR, without jackbean meal served as control. These diets were fed to Tilapia *Oreochromis niloticus* fingerlings (6.37 ± 0.07 g) in a glass tanks for 5 weeks. Faecal collection was made by siphoning after four hours of each feeding. Proximate analysis of both diets and faecal samples were carried out for crude protein, lipid, crude fiber, energy, and AIA. These were used to calculate the digestibility coefficient of nutrients. There was no significant difference ($p > 0.05$) in lipid and organic matter digestibility of the fish fed control diet and test diets. Significant ($p < 0.05$) variations occurred in nitrogen-free extracts, protein and crude fiber digestibility of fish fed control diet and the test diets.

Key words: Digestibility, *in vivo*, jackbean, *Oreochromis niloticus*.

INTRODUCTION

Soybean meal has high protein content and the best protein quality among plant protein feedstuffs used in fish feeds (Davies et al., 1999). It has been reported to partially or totally replace fishmeal in diets of many aquaculture species (Lovell, 1988; Lim and Akinyama, 1992). However, due to various other uses, the seed meal is now scarce and even expensive, beyond the reach of fish farmer. It then becomes a priority to look for cheaper, alternative protein source. Jackbean, *Cannavalia ensiformis* (L.) DC, an indigenous legume has shown potential as protein and energy sources of fish diets (Oliveira-Novoa, 1988) because of its high seed yield, energy and protein content. It has up to 30% crude protein and 60% carbohydrate (Udedibie, 1990). Digestibility of nutrients in fish diets needs to be studied because it is the digested feed, which is absorbed, that is made available for cellular metabolism. The resultant of which will be tissue synthesis, and repair of worn-out tissues and various energy utilization channels (Yudkin, 1985; NRC, 1993). The most important characteristics of feedstuffs are the bioavailability of nutrients; hence reliable data on different ingredients for each species need to be well considered as a necessary prerequisite (Jauncey, 1993; Fagbenro et al., 2003). Borghesi et al. (2008) reported that knowing nutrient digestibilities of feed ingredients elicit interchangeability of feed ingredients without reducing animal performance. De Silva and Anderson, (1995) also observed that it is essential to have a knowledge of the digestibility of the main ingredients as well as of the whole diet in feed formulation and manufacture; In combination, chemical analysis and apparent digestibility coefficient (ADC) results allow us to precisely estimate not only the contribution of a particular protein source to a complete fish feed but also how much feed wastes and undigested nutrients (faeces) will potentially accumulate in fish pond (Koprucu and Ozdemer

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Table 1. Gross composition (g/100 g dry matter) of experimental diets fed to *O. niloticus* at varying replacement levels of Jackbean meal.

Feed ingredient	Control	Test diets			
	CTR	D10	D20	D30	D40
Fish meal	24.00	24.00	24.00	24.00	24.00
Jackbean meal	--	6.00	12.00	18.00	24.00
Soyabean meal	32.00	30.00	26.00	22.00	19.00
Maize	24.00	22.00	20.00	18.00	15.00
Cod-liver oil	2.50	2.50	2.50	2.50	2.50
Vegetable oil	3.50	3.50	3.50	3.50	3.50
Vit/min premix	2.00	2.00	2.00	2.00	2.00
Cassava Starch	10.00	10.00	10.00	10.00	10.00

2005). Various direct and indirect methods have been used to study digestibility. The direct method involves measuring all the feed consumed by the fish and all of the resulting excreta. A measured amount of feed and the faeces collected are analyzed for their nutrient content. The amounts of the nutrients in the excrements are then subtracted directly from those in the feed to determine the amounts retained (NRC, 1993). Smith (1971, 1976) and Smith et al. (1980) used an aquatic modification of the metabolism chamber designed for terrestrial animal studies; it allowed for the separate quantitative collection of gill, urine, and faecal excretions of rainbow trout. The fish were force-fed a measured amount of feed, and the various excrements were subsequently collected and analyzed for their nutrient content. The indirect method involves the use of a non-digestible exogenous or endogenous marker/indicator. It is assumed that the amount of marker in the feed and faeces remains constant throughout the experimental period and that all of the ingested markers appear in the faeces. The digestibility of the nutrient in question can then be determined by assessing the difference between the feed and faecal concentrations of the marker and the nutrient (NRC, 1993). Digestion coefficients determined by the indirect method have been useful and feeding regimens based on these data have been successful (Takeuchi et al., 1979; Cho and Kaushik, 1985; Wilson and Poe, 1985; Mangalik, 1986; Satoh et al., 1992). Fish can be sacrificed and the faecal samples either (i) removed from the lower large intestine (Smith and Lovell, 1971), or by (ii) gentle pressure in the abdomen of the fish (Nose, 1960a, b), or by (iii) suction method can be used (Windell et al., 1978). Faecal samples can also be periodically removed from the tank by siphoning or using settling column (Smith, 1989). Cho and Slinger (1979) have shown that if excretions are removed from the fish tank soon after expulsion, the collection of passively excreted faeces can give good digestibility data. Hence an attempt was made to evaluate *in vivo* apparent digestibility coefficient of nutrients in processed jackbean meal using acid insoluble ash as indicator.

MATERIALS AND METHODS

Source of *C. ensiformis* seeds

The jack bean seeds were obtained from the National Root Crops Research Institute (NRCRI) in Vom, Plateau State, Nigeria. They were broken into coarse pieces (about 3 to 6 parts) using a grinding machine. The broken seeds were subjected to one hour ordinary cooking and the products was dried in the sun (30 to 32°C) for three days, milled and analysed for its proximate composition.

Five isonitrogenous diets (30% crude protein) were formulated as depicted in Table 1. The soybean meal protein in the diets was substituted at a rate of 10, 20, 30, 40% to serve as test diets designated as D10, D20, D30, and, D40 respectively. A diet, called as diet CTR, with soybean meal and without jack bean meal served as a control. The feedstuff was milled using grinding machine. Hot water was added to aid binding and then introduced into Hobart A-200T pelleting and mixing machine to obtain a homogenous mixture and then transferred through a mincer to produce 0.6 mm (long) 0.2 mm (diameters) pellets which were immediate sun dried (30 to 32°C). After drying for 3 days, the diets were kept in a freezer (-20°C).

Culture condition

Nile tilapia (*Oreochromis niloticus*) fingerlings (6.36 ± 0.07 g) were stocked in 15 glass aquarium tanks at the Department of Fisheries and Wildlife, Federal University of Technology, Akure, Nigeria. Fish were acclimated to laboratory conditions for seven days, during which they were fed a 30% crude protein diet before the experiment started. Fish were not fed for 24 h before the onset of the experiment. Triplicate batches of fish were fed to satiation twice a day (08:00 to 09:00 and 16:00 to 17:00 h) on each of the five experimental diets for an initial seven days before fecal collection began. Collection of faeces samples were carried out for 14 days by siphoning using a pipe (2 cm diameter) three hours after feeding. Uneaten diet was siphoned out 20 min after feeding. Droppings from the same tank were pooled together in a bowl and stored in freezer. The amount of dissolved oxygen (using a YSI model 57 oxygen meter) water temperature and pH (using electronic pH meter) were measured daily in all tanks.

Proximate analysis

The proximate analysis of the protein feedstuffs, diets and faecal matter samples were carried out in three replicates using the procedures described by the AOAC (1990). Moisture content was

Table 2. Proximate composition (%) of experimental diets fed to *O. niloticus* at varying replacement levels of Jackbean meal.

Feed ingredient	Control	Test diets			
	CTR	D10	D20	D30	D40
Protein	30.18	30.19	30.24	30.55	30.88
Lipid	10.39	10.07	9.76	10.52	10.41
Fiber	5.58	5.62	5.65	5.7	5.69
Ash	6.04	5.09	5.41	5.26	5.3
NFE	39.56	40.08	40.84	39.61	38.96
Moisture	8.25	8.95	8.1	8.36	8.76
Energy (Kcal /Kg)	430.89	430.06	430.53	431.42	432.83
AIA (%)	3.05	3.18	3.08	3.11	3.36

NFE-Nitrogen free extract, AIA- Acid insoluble ash.

determined as a percentage loss in weight after drying a known weight of the sample at 100°C until constant weight was obtained. Crude protein was determined by digesting a known weight of the sample (w_1) in Kjedahl flask unit using 25 ml of concentrated H_2SO_4 and a gram of copper catalyst. The percentage nitrogen was then calculated as;

$$\% \text{ Total Nitrogen (N)} = \frac{T_1 \times M_2 \times 0.014 \times V_1}{W_1 \times V_2} \times 100$$

T = Titre Value
Ma = Molarity of acid
W_1 = Weight of the sample
V_1 = Initial Volume put in a distillation unit
V_2 = Final volume Obtained from the distillation

% Crude Protein = N x 5.4 (Legume)

A factor value of 6.25 was used to convert nitrogen to protein for animal protein. The lipid content was determined by subjecting the sample to a continuous extraction with petroleum ether using Soxhilet apparatus. The residue from ether extraction was subjected to successive treatment of boiling with dilute acid and dilute base, after which it was filtered and ignited in a furnace. The difference obtained between the burnt and unburnt fractions was calculated to be crude fibre. Ash Content was determined by subjecting the already dried samples with known weight to ignition in a muffle furnace at 550°C for 8 h. The nitrogen free extract was estimated by difference. Energy contents of faeces and the diets were determined using an adiabatic bomb calorimeter.

Acid insoluble ash (AIA) analysis

AIA analyses were carried out on the diets and feces. AIA was obtained by adding 25 ml of 10% HCl to the weighed ash content of a sample. This was covered with a water-glass and boiled gently over a low flame for five minutes. This was then filtered using ashless filters and washed with hot distilled water. The residue from the filter was returned to the crucible and ignited until it was carbon free after which it was weighed. Percentage AIA was calculated as;

$$\%\text{AIA} = \frac{\text{Weight of AIA}}{\text{Weight of Ash}} \times 100$$

Determination of digestibility coefficient

This was calculated on the percentage of AIA in feed and in faeces and the percentage of nutrient on diets and feaces

$$\text{Apparent Organic matter Digestibility (\%)} = 100 - 100\frac{\text{AIA in Diets}}{\text{AIA in Faeces}}$$

$$\text{Apparent Digestibility (\%)} = 100 - 100\frac{(\text{AIA in Diets})}{(\text{AIA in Faeces})} \times \frac{(\text{Nutrients in Faeces})}{(\text{Nutrients in Diets})}$$

Statistical analysis

All determinations were conducted in triplicates and the means were subjected to Analysis of Variance, where, the ANOVA revealed a significant difference, Fishers Least Significant Difference (LSD) was used to compare differences among individual treatment means using SPSS version 13.

RESULTS AND DISCUSSION

The ingredient and proximate composition of experimental diets is presented in Tables 1 and 2 respectively. The result revealed the diets were isonitrogenous and isocaloric conforming to the recommendation of optimum dietary protein of the fish (Luquet, 1991). The lipid requirement of the fish also met the standard of the aquadiets (Jauncey and Ross, 1982). Acid Insoluble Ash (AIA) was used as a marker because it is more reliable indicator of digestibility coefficient (Halver et al., 1993) since the dietary ingredient (ash) is used and analysis of this component in feces collected uses simple gravimetric technique. Defecation of the fish started about four hours after feeding and lasted for 15 to 25 min. Same observation was made by Adeparusi and Jimoh (2002).

Table 3 revealed the proximate composition of the faecal sample. A general reduction in the nutrients of the faeces as compared to the diets showed that some percentages of nutrients were absorbed and made available for metabolism. There was a significant

Table 3. Proximate composition (%) of faecal samples of *O. niloticus* fed varying replacement levels of Jackbean meal.

Parameter	Control	Test diets			
	CTR	D10	D20	D30	D40
Protein	8.82[b]	10.64[b]	10.97[b]	12.01[ab]	14.01[a]
Lipid	3.11	3.02	3.05	2.74	3.3
Fibre	3.92[a]	4.1[a]	4.16[a]	4.31[a]	4.48[b]
Ash	12.4[a]	14.04[a]	14.11[a]	14.12[a]	13.98[b]
NFE	60.8	60.19	57.8	56.94	54.18
Moisture	10.95	8.01	9.91	9.88	10.05
Energy Kcal /Kg	328.5	333.54	327.78	327.2	332.48
AIA (%)	8.9	8.95	9.78	9.12	8.94

Table 4. Apparent digestibility coefficients of the nutrients in the diets fed to O. niloticus.

Parameter	Control	Test Diets			
	CTR	D10	D20	D30	D40
Crude protein	89.98[a]	87.48[ab]	88.57[ab]	86.59[b]	82.96[c]
Crude fibre	74.88[a]	74.69[a]	76.79[a]	74.26[a]	70.38[b]
Lipid	89.7	89.28	90.08	91.13	87.97
NFE	55.36[a]	50.98[b]	46.62[c]	46.65[c]	47.77[c]
Energy	76.02[a]	74.32[b]	73.87[b]	72.28[c]	71.12[c]
Organic matter	64.67	63.26	67.33	65.52	61.53

difference ($p < 0.05$) in the crude protein, ash and nitrogen free extract of the faecal samples tested. Diet D4O had the highest fecal nitrogen. The result agreed with Adeparusi and Jimoh (2002) that there was a reduction in nutrient value of faecal sample of *O. niloticus* fed lima bean (*Phaseolus lunatus*) when compared to the diets.

Table 4 presented the apparent digestibility coefficient of nutrients in the diets fed to *O. niloticus* fingerlings. A significant difference ($p < 0.05$) was observed between different nutrients in the different diets. The results of the apparent protein digestibility of the seedmeal are similar to that reported in Fagbenro (1988) for the same seed meal fed to *O. niloticus* but lower than the values reported by Martinez-Palacios et al. (1988) who fed the same seed meal to *O. mossambicus*. The little variation observed could be attributed to variability of nutrients as well as differences in nutrient processing, experimental methodology and feces sampling technique (Jauncey, 1993).

No significant difference ($p > 0.05$) occurred for crude fibre digestibility of *O. niloticus* fed jackbean meal at varying replacement level except for diet D40; the crude fibre digestibility coefficient in this study was higher than the carbohydrate digestibility in the same experiment. This could be attributed to the natural feeding habit of tilapia that consists mainly of plant material (Pullin, 1983). *O. aureus* was found to digest highly fibrous feedstuffs such as alfalfa meal (Mgbenka and Lovell, 1987). Crack and cook processing method improved the digestibility of crude fibre. This result conforms to the report of Adeparusi and Jimoh (2002) that fibre digestibility of *O, niloticus* fed lima bean diet was improved with toasting and autoclaving. No significant ($p < 0.05$) variation was observed in the lipid digestibility of *O. niloticus* fed with processed jackbean meal. The high lipid digestibility by this species was found to be in line with what was reported in Hossain et al. (1992) for rainbow trout. A range of 76 to 97% fat digestibility of various sources of fat has been reported for channel catfish (Lovell, 1977). Andrew et al. (1978) reported that the ability to digest fat appears to be influenced by temperature and the level of fat in the diet.

There was a significant difference ($p < 0.05$) in the carbohydrate digestibility of *O. niloticus* fed processed jackbean meals and the control, however there was no significant difference ($p > 0.05$) in the carbohydrate digestibility of the fish fed diet CTR, DIO and D40. The low carbohydrate digestibility recorded in this study was similar to that reported by Adeparusi and Jimoh (2002) for *O. niloticus* fed lima bean. The result however deviated from the report of Popma (1982) that warm water fish like *O. niloticus* absorbs 60% or more of raw corn starch. The digestibility of carbohydrates has been shown to vary

Table 5. Mean value of water quality parameters in the experimental tanks.

Parameter	Control	Test diets			
	CTR	D10	D20	D30	D40
Temperature (°C)	26.20±0.21	26.40±0.73	26.58±0.32	26.10±0.15	26.35±0.32
Dissolved oxygen (g/dm^3)	6.78±0.29	6.55±0.32	6.56±0.18	6.59±0.54	6.79±0.64
pH	7.11±0.35	6.98±0.21	7.20±0.11	6.92±0.53	7.04±0.27

with their complexity, source treatment and level of inclusion in the diet (Phillip, 1972; Lovell, 1977; Cho and Slinger, 1979). Generally tilapia have been reported to have ability to digest carbohydrates relatively well in feedstuff but not as well as fat or protein Lovell (1988). There was significant difference ($p < 0.05$) in the energy digestibility of *O. niloticus* fed processed jackbean meals and the control. However, there was no significant difference ($p > 0.05$) in the energy digestibility of the fish fed with D20 and CTR and the fish fed with D10 and D40. The high apparent digestibility coefficient of energy recorded in this study is similar to the values recorded by Fagbenro (1998) of the same seed meal fed to *O. niloticus*. Lovell and Durve (1982) revealed that cooking improved gross energy digestibility.

These were no significant difference ($p > 0.05$) in the organic matter digestibility of *O. niloticus* fed processed jackbean meals and the control. The organic matter digestibility coefficient reported in this study is similar to that of Fagbenro (1998) and Olivera et al. (1988), but lower than the values reported by Martinez-Palacios et al. (1988). The variation may be attributed to processing methods and or experimental methodology. The crack and cook processing technique applied to jackbean meal improved its digestibility.

Table 5 revealed the physico-chemical conditions under which the experiment was conducted. The values were within the optimum range for the normal physiological functioning of not only tilapia but also most of the warm water fish species (Boyd, 1990). Lazo and Davies (2000) reported that experimental culture system must be suitably designed to allow maximum growth of fish and provide adequate water quality throughout the experiment. Conclusively, this study reveals that up to 20% of soybean meal in the diets of *O. niloticus* can be replaced by jackbean meal without negatively affecting the fish performance vis-à-vis the rate at which the nutrients are absorbed in the species. This will reduce the cost of fish feed considerably.

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