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Effect of Faecal Collection Methods on Nutrient Digestibility in *Oreochromis niloticus* Fed Soya Bean Diets

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Abstract: A 30% crude protein control diet was formulated for the Nile Tilapia, *Oreochromis niloticus* with fishmeal as the main protein source. The fishmeal content was replaced with soaked and cooked soybean at 25, 50, 75 and 90% equi-protein levels. Diets were designated as D1, D2, D3 and D4 respectively. *Oreochromis niloticus* fingerlings (mean weight, 10g) were randomly distributed at 20 fish per tank in duplicates. After seven days of acclimation, the fish were fed on the experimental diets for 21 days. Faecal collection was carried out using five different methods: siphoning directly from the aquaria, stripping by applying pressure on the intestinal section and collection of samples from three sections of the intestine: upper region, middle region and lower region. Acid insoluble ash was used as internal marker to assess digestibility of crude protein, lipid, ash and carbohydrate (NFE). In the siphoned, stripped and middle intestine collection methods fish fed diet 2 had the highest digestibility coefficients in crude protein, lipid, ash and NFE, while in the upper and lower intestine the control diet had the highest digestibility coefficients in crude protein, lipid and NFE. In samples collected from the lower intestine diet 4 had the least digestibility of all nutrients. While n the upper intestine fish on diet 3 had the least nutrient digestibility except in NFE. Diets 3 equally had the least digestibility in the siphoned diets except in NFE. In crude protein fish fed diet 2 had the highest digestibility of nutrients while those on diet 3 had the least. Highest numerical quantification of digestibility occured in the siphoned method while the least was in the stripped. This study shows that soybean inclusion levels and sampling methods have significant effects on the coefficients of digestibility.

Key words: Digestibility, coefficient, faecal collection methods, Soybean, diets, *Oreochromis niloticus*

INTRODUCTION

Due to increase in intensive culture of many species of fish there is a corresponding increase in demand for more efficient diets. Nutritionally or physically faulty feed impairs fish productivity and has substantial impact on the environment through accumulation of wasted feed. There is therefore the need to produce least cost diet having optimal nutritional standard. The nutritional quality of a feed or ingredients is based on the availability of its nutrient to the animal. This is assessed through digestibility of such feed by the animal. In order to evaluate a feed digestibility coefficients are used as a general measure of the nutritive value. A feedstuff may appear from its chemical composition to be an excellent source of nutrients but may be of little actual value unless it can be digested and absorbed. Growth response in addition to digestibility of nutrients contained in feedstuffs could be used as bases for determining the suitability of their use in fish feeds[1]. According to Rodrigues[2] there is lack of information on the digestibility of the different components of feedstuffs used in fish feed. Such data is required for efficient diet formulation. Digestibility studies therefore contribute to the optimization of nutrient utilization and consequently lower energy losses. Such studies would reduce loss of nutrients into the environment.

Faecal collection in fish still poses a serious technical problem that affects the accuracy of reported digestibility results. Several methods have been used in collecting faecal dropping: dissection of digestive tract, siphoning and stripping and direct method of total faecal collection. All these methods have been found to have inherent errors[2]. Digestibility of feed/ingredient could be measured directly by comparing the quantity of feed ingested with the quantity egested. Indirect method makes use of a marker, which is an indigestible material, either introduced in small quantity, e.g. chromium oxide or those present in the diet e.g. Acid-Insoluble Ash (AIA). Chromic oxide has been the most extensively used digestibility indicator. Acid-insoluble ah is mainly silica. It is found as a component of the feed that makes it less costly. It has been used in digestibility determination in monogastric animals. AIA ratio technique gives almost identical mean digestibility values with conventional total faecal collection methods in fish[3].

Soybean is regarded universally as the most attractive plant protein source for animal feed[4] with the best amino acid profile and essential amino acid requirements of aquatic species. Soybean meal is one of the most acceptable alternatives and less expensive source of good quality protein used in fish to replace the high cost good quality fish meal used in traditional fish diet.

This study compares the digestibility of fishmeal and soybean diets at different gut regions and in the droppings of *Oreochromis niloticus.*

MATERIALS AND METHODS

Fish: Nile Tilapia fingerlings (average weight, 10g) obtained from ADP fish farm, Akure was used for the trials.

Diets: Five experimental diets were formulated; the control diet (CTR) has 0% soybeans. The other 4 diets i.e. D1, D2, D3 and D4 contained 25, 50, 75 and 90% soybean meal protein as a replacement for fishmeal (Table 1). Oil content was varied to balance the energy content of each diet. Feed components were weighed, thoroughly mixed. Starch was homogenized and added to bind components together. Pelleting was done using Hobart mixer and pelleter with 3mm diameter die size.

Experimental procedure: The experimental was carried out in glass tanks of 70 x 40cm (L x B x H) with 50 litres capacity each. A total number of 200 fingerlings were distributed randomly into the 10 tanks and labeled as control. (CTR), Diet 1 (D1), Diet 2 (D2), Diet 3 (D3), Diet 4 (D4) and each was duplicated. The fish was acclimatized for seven days fed on the control (CTR) diet. The fingerlings were fed twice daily between 8.00a.m. - 9.00a.m. and 4.00p.m. – 5.00p.m. for three weeks at 3% body weight.

Sample collection and analysis

Siphoning: Two hours after feeding, the faecal output of the fish from each diet was siphoned out into sieve immediately after expulsion, faeces were air-dried and stored in the deep freezer through the experimental period, before the analysis.

Stripping: Faecal samples were collected by applying pressure anteroposteriorly on the abdomen of the fish[5] . Faecal droppings for each group were pooled together, dried in an oven at 55°C and used for digestibility estimation.

Table 1: Gross composition of soybean diets fed to *O. niloticus* (%)

	Diets at different Fishmeal: Soybean substitution rates				
	CTR				
	(100:00)	D1 (75:25)	D2 (50:50)	D3(25:75)	D4 (10:90)
SBM	-	11.36	22.27	34.06	45.71
Corn	38.71	38.71	38.71	38.71	38.71
Starch	1.00	1.00	1.00	1.00	1.00
Salt	0.05	0.0	0.05	0.05	0.05
Oil	1.00	2.26	3.52	4.78	6.05
BM	3.00	3.00	3.00	3.00	3.00
Vit premix	3.00	3.00	3.00	3.00	3.00
Rice bran	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100.00

FM Fishmeal
SBM Soybean meal (soaked and cooked)
BM Bone meal

Gut sectioning: Fish were killed by a blow to the head. The entire digestive tract of fish on all diets was removed. Gut was divided based on visual observation of lengths into three sections: upper one-third, middle and lower one-third of the intestine. The content from each region was collected and analysed. Guts were sectioned in accordance with the methods of Hastings[6].

Digestibility coefficient in all collection methods was calculated using the formular of Maynard *et al.,*[7]:

$$\text{Digestibility (\%)} = \frac{100 - 100\,[\%\,\text{AIA in feed} \times \%\,\text{nutrient in faeces}]}{\%\,\text{AIA in faeces}\ \%\,\text{nutrient in feed}}$$

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

Chemical analysis: Feed and faecal samples obtained from the digestibility trials were analysed according to the methods of AOAC Standard Methods of the Association of Official Analytical Chemist (AOAC, 1990). Carbohydrate was calculated by difference. Acid Insoluble Ash (AIA) was determined according to the method of Buddington[8].

Statistical analysis: Differences between the mean test groups were established by analysis of variance (ANOVA). The Duncan test was used to compare differences[9].

RESULTS

A comparison of crude protein digestibility of fish in the different faecal collection methods is given in Table 2. Digestibility of protein was generally high in all methods of faecal collection. Digestibility coefficients of crude protein ranged from 74.80 to 80.91% in the siphoned sample, 83.22 to 71.65% in the stripped, 63.60 to 72.28% in upper intestine, 66.16 to 84.82% in the

Table 2: Digestibility coefficients of protein from different faecal collection methods in *O. niloticus* fed soybean diets

	Diets				
Feacal collection methods	Control	D1	D2	D3	D4
Siphoned	74.80±2.26	78.30±2.49	80.81±0.61	75.52±3.14	80.57±0.10
Stripped	65.97±2.16	63.28±2.01	71.65±3.41	63.22±2.29	63.26±2.24
Upper Intestine	70.08±2.77	66.16±2.66	72.28±2.55	63.60±1.70	68.04±2.80
Middle Intestine	81.00±2.63	73.27±2.25	84.82±2.26	66.16±2.74	68.26±2.77
Lower Intestine	80.80^{a}±0.96	73.96^{ab}±1.05	79.12^{a}±1.39	69.47^{ad}±2.25	66.88^{ad}±1.70

a, b, c means with different superscripts within a column are significantly different (p<0.05)

Table 3: Digestibility coefficients of lipid from different faecal collection methods in *O. niloticus* fed soybean diets

	Diets				
Feacal collection methods	Control	D1	D2	D3	D4
Siphoned	73.90^{b}±1.16	72.10^{bc}±2.83	80.68^{a}±2.32	65.80^{e}±	71.29^{bd}
Stripped	72.53^{a}±2.56	59.50^{b}±1.92	73.75^{a}±2.70	5.48^{bc}±0.85	54.97^{bd}±2.45
Upper Intestine	78.56^{a}±2.55	63.34^{b}±1.64	74.59^{a}±2.67	55.00^{bd}±1.64	57.70^{bd}±2.21
Middle Intestine	83.25^{a}±2.28	79.54^{b}±1.33	87.24^{a}±1.70	64.75^{cd}±2.25	68.72^{c}±2.26
Lower Intestine	84.16^{a}±1.32	81.40^{b}±1.51	85.36^{a}±2.03	69.04^{b}±1.58	68.32^{bc}±1.24

a, b, c means with different superscripts within a column are significantly different (p<0.05)

Table 4: Digestibility coefficients of ash from different faecal collection methods in *O. niloticus* fed soybean diets

	Diets				
Feacal collection methods	Control	D1	D2	D3	D4
Siphoned	64.00±2.83	49.78±3.37	69.410±2.25	37.00±2.26	53.89±
Stripped	54.49±2.56	15.76±2.46	62.20±2.83	25.27±2.22	8.80±0.28
Upper Intestine	61.60±1.75	48.97±1.47	65.35±2.11	43.54±1.77	44.07±2.76
Middle Intestine	69.25±2.28	66.01±2.84	72.94±2.77	47.83±2.02	48.02±2.83
Lower Intestine	72.16±1.30	68.07±1.71	74.32±2.40	41.95±1.54	22.24±1.13

a, b, c means with different superscripts within a column are significantly different (p<0.05)

Table 5: Digestibility coefficients of carbohydrate from different faecal collection methods in *O. niloticus* fed soybean diets

	±Diets				
Feacal collection methods	Control	D1	D2	D3	D4
Siphoned	68.20±2.97	64.97±3.01	74.70±2.55	60.76+2.66	69.84±1.70
Stripped	44.24±2.57	35.20±3.06	56.26±2.81	30.57±0.01	35.59±0.40
Upper Intestine	56.16^{a}±2.83	38.90^{b}±1.98	55.12^{a}+3.20	28.24^{d}+0.28	38.44^{bc}±2.86
Middle Intestine	66.25±2.18	51.16±3.45	68.98+2.77	38.43+2.36	34.08±2.73
Lower Intestine	66.64^{a}±1.10	53.81^{a}±1.57	65.92^{a}+2.66	42.38^{ab}+2.66	36.16^{ac}±0.28

a, b, c means with different superscripts within a column are significantly different (p<0.05)

Table 6: Range of nutrient digestibility in different methods of faecal collections *O. niloticus* fed soybean diets

	Digestibility coefficient range					Mean ranges	
Nutrients	siphoned	stripped	Upper intestine	Middle intestine	Lower intestine	Lower range	Upper range
Crude protein	74.80-80.91	63.22-71.65	63.60-74.08	66.16-84.82	66.88-80.80	66.93 (4.7)	78.45 (4.9)
Lipid	65.80-80.68	54.97-73.75	63.60-78.56	66.16-87.24	68.32-84.16	63.77 (4.7)	80.88 (4.6)
Ash	37.00-69.41	25.27-62.20	43.54-65.35	47.83-72.94	36.16-74.32	37.96 (7.1)	68.84 (4.6)
NFE	60.76-74.70	30.57-56.26	28.24-56.16	34.08-68.98	36.16-66.64	37.96 (11.7)	66.64 (7.3)

middle intestine and 66.88 to 80.80% in samples from the lower intestine. It was only in the lower intestine that significant difference (p<0.05) occur in the digestibility of crude protein from the different diet. Among the diets, fish fed diet 2 had the highest crude protein digestibility coefficients among the collection methods while fish on the control diet had the least. Protein digestibility coefficient was highest in faecal sample from the middle intestine while the least was from stripped faecal sample. The least protein digestibility was in diet 3.

Table 3 shows the result of the apparent digestibility coefficient of lipids in all the faecal collection methods. There were significant differences (p<0.05) in the digestibility of lipid from the different diets. Digestibility coefficient of lipid was highest in the middle intestine and least in the stripped sample. In most collection methods fish fed diet 2 had the highest lipid digestibility except in the upper intestine where the highest was on fish fed the control diet. Least value in lipid digestibility was obtained in fish fed diet 3.

Apparent ash digestibility coefficients in all the faecal collection methods are as shown in Table 4. Unlike in lipid there were no significant differences ($p>0.05$) in the digestibility of ash from the different diets. The highest digestibility coefficient in ash was obtained in the lower intestine in fish fed diet 2 while the least was also recorded in the lower intestine of fish fed diet 4.

Carbohydrate digestibility of fish in the different faecal collection methods is given in Table 5. Significant differences ($p<0.05$) in the digestibility occurred only in the upper and the lower intestine of fish. Highest carbohydrate digestibility was in siphoned sample while the least was in the samples from the upper intestine of fish fed diet 3.

Table 6 shows the digestibility coefficients range in nutrients in *O. niloticus* fed on the diets. Faecal sample collected through siphoning has the highest apparent digestibility values than those obtained by either manual stripping or intestinal dissection. Stripping gave the least numerical quantification of digestion among all the methods used. The siphoned and middle intestine samples gave the highest values. Samples from the upper and lower regions gave values relatively in-between the two.

Based on frequency of occurrence in all methods of collection diet 2 appears to be the diet with highest digestibility of nutrients followed by the control while diet 3 had the least.

DISCUSSION

Nutrients were absorbed from the different sections of the intestine. This agrees with the work of Smith and Lovell[10] who reported similar observation in the channel catfish. This would mean that the required enzymes for digestion are distributed throughout the gut. Olatunde (1983) reported the presence of pepsin in the intestine of *S. galileus*. This enzyme is found only in the stomach of most vertebrates. Enzymes responsible for the digestion of lipids (lipases), carbohydrates (amylases) and proteins (proteases) have been reported in the pyloric caeca or intestinal mucosa of fishes[11]. Trypsin, chymotrypsin, amylase and esterase activities have been measured from the intestine of fish[2]. Trypsin, chymotrypsin, amylase and estrase activities have been measured from intestine of Tilapia[12,13]. Oseman and Caceci, suggested from their study on the stomach of *O. niloticus* that the gastric epithelial cells are specialized for the absorption of easily digested substances such as carbohydrates and short-chain fatty acid.

Fat was found to be mostly absorbed in the lower two-thirds of the intestine of *O. niloticus*. This shows a marked difference from what was reported for carp where absorption of nutrients in this study was found to be highest in the lower two-thirds of the intestine[14]. High lipid digestibility in *O. niloticus* shows that lipid from soaked-cooked soya bean is well utilized. This is a good phenomenon since lipid can also have a sparing action on protein. High digestibility of crude protein showed that soybean could replace part of the fishmeal in *O. niloticus* without compromising quality. Soybean has one of the best amino acid profiles among vegetable proteins in meeting the essential amino acid requirements of fish. Digestibility of carbohydrate was high in fish fed diets 2 irrespective of the faecal collection method used. It was equally fairly high in the other diets although values were low in diets 3 and 4. The ability of *O. niloticus* to digest carbohydrate has been well recorded in literature. This would lead to maximum utilization of available protein as a result of the protein sparing-action of carbohydrate. Nile Tilapia being an omnivore digests carbohydrate efficiently[15]. Digestion of carbohydrate and protein was more in the lower portions of the intestine. This is in contrast to the report of Bowen[16] who reported that protein digestion was completed in the first quarter of the intestine of *O. mossambicus*.

Digestibility of crude proteins and lipids were found to be higher than 50% in *O. niloticus*. This could be due to the fact that all ingredients were sieved through a fine mesh thereby producing a small particle size (and the pellet size was small diameter of 3mm) for the fish to seize and eat. Solomon and Bradfield[17] found out that as meal size increases, absorption efficiency decreases. Digestion could have been further enhanced through soaking of soybean before cooking. Lof[18] stated that these two processes removed the bitter (beany) taste and reduced anti-nutritional factors. The presence of moisture during heating could have enhanced the inactivation of trypsin inhibitor.

Digestion of mineral was comparatively low in all faecal collection methods. Fish do not need high quantity of minerals from their diets. The highest (diet 2) and the least (diet 4) ash digestibility were both recorded from the lower intestine. This shows that the type of diet affect the rate of digestion at different zones of the gut.

Highest apparent digestibility values were in faecal sample collected through siphoning. This may not be due to leaching of nutrients because the experiment was monitored such that faeces were siphoned immediately after expulsion. This could have prevented nutrients leaching. Nutrients from siphoned faeces could have been leached into water[2] but the rate of such leaching may not be appreciable in this study. Also faecal droppings of *O. niloticus* were in strands coated with protein-like subsistence which prevent faeces from being dispersed into water. This made collection of faeces by siphoning easier. It also reduces the level of faeces contamination with uneaten feed. Siphoning in this study could therefore be considered as a dependable estimate of digestion. Therefore collection through siphoning could be adopted. This is because this method will not necessitate killing of the fish nor applying pressure, which is stressful[19].

The result also showed that digestibility coefficient of the faeces collected through manual stripping and intestinal dissection has lower digestibility coefficients. It is the most conservative in measuring the rate of digestion. Although collection of faecal samples by abdominal stripping would have been expected to be similar to samples from the rectal lower intestine. This could be because manual stripping involves applying slight pressure to the abdominal cavity between the pelvic fins and the vent. During this process the body fluids of intestinal epithelium are stripped along and it may contaminate the faeces. Stripped faecal material could also have come from sections other than rectal region. In collecting the faecal sample through dissection, undigested food may be collected along with the faeces, this could also result into underestimation of its digestibility.

Given the increasing interest in replacing fish meal component of production type diet with cheaper alternative protein sources there is an obvious need for more information to establish whether such manipulation would affect the efficient digestion and absorption of nutrients. Thus study shows that the faecal sampling methods have significant effects on the coefficients of digestibility. Digestion was found to be different at different sections of the gut. Inclusion levels of soybean affected were found to affect nutrient digestibility. A ratio of 1:1 soybean protein to fish meal protein gave a higher nutritionally blend than others. This was depicted by the digestibility of all the nutrients in fish fed diet 2.

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