

Digestibility of Soybean Cake, Niger Seed Cake and Linseed Cake in Juvenile Nile Tilapia, *Oreochromis niloticus* L.

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Abstract

The apparent digestibility coefficients (ADCs) of dry matter, protein, lipid and energy for soybean cake (SBC), Niger seed cake (NSC) and linseed cake (LSC) were determined in juvenile Nile tilapia. The ADCs were determined using faeces collected with a settling chamber attached to the fish rearing tank. Test diets contained 70% reference diet and 30% test ingredients, with Cr₂O₃ as an inert marker. All treatments were triplicated. There was significant difference in Apparent Dry Matter Digestibility (ADMD), Apparent Protein Digestibility (APD) and Apparent Energy Digestibility (AED) between the test ingredients. However, there was no significant difference (P>0.05) in Apparent Lipid Digestibility (ALD) between the test ingredients. Of the three ingredients tested, SBC produced the highest nutrient digestibility coefficients (P<0.05) while LSC showed the lowest nutrient digestibilities (P<0.05). The NSC, which was the cheapest plant protein source, was a good feed ingredient for Nile tilapia diets in terms of overall nutrient composition and acceptable digestibility coefficients enabling more accurate and economical feed formulation.

Keywords: Juvenile nutrition; Plant proteins; African aquaculture; Fish meal; Ethiopian oilseeds

Introduction

With the increase in intensive aquaculture, demand for more efficient aquafeed is rising. Feed comprises the principal operating cost in fish production and the main protein source has traditionally been fish meal [1]. Fishmeal, the conventional protein source in aquaculture feeds, supports good fish growth because of its protein quality and palatability [2]. However, fish meal is often scarce and expensive, due to limited availability and high demand, which often leads to high fish production costs [3,4]. According to Ng and Romano [2], cost-effective, practical aquaculture feeds can be produced without the use of fish meal with no resulting or apparent loss in fish growth in some species. Hence, replacing fish meal with cheaper ingredients of either animal origin or protein-rich plant sources is a necessary priority for nutrition research [1,2]. In view of this, oilseed meals have been found to have considerable economic potential [2,5]. While grain legumes have not been widely used within aquaculture feeds, oilseeds and their by-products frequently constitute a major source of dietary protein within aquaculture feeds for warm water fish species such as those commonly used in African aquaculture, including tilapias (*Oreochromis* spp.) and African catfish [6].

A feed ingredient may appear from its chemical composition to be an excellent source of nutrients but will be of little actual value unless it can be ingested, digested and absorbed in the target species. Only a proportion of ingested food is digested and its nutrients absorbed, the rest is voided as faeces. By definition, digestibility is a relative measure of the extent to which ingested food and its nutrient components have been digested and absorbed by the animal. Knowledge of nutrient digestibility is, therefore, important to establish the potential of an ingredient for use in diets of aquaculture species [2,7]. Determining the digestibility of nutrients in feedstuffs is important not only to enable formulation of diets that maximize the growth of cultured species, by providing appropriate amounts of available nutrients, but also to limit the wastes produced by the fish and reduce costs [1,2,7,8].

For tilapia feeds typical protein sources examined have included cereal grain products [9], defatted soybean meal, full-fat toasted

soybean, lupin seed meal and faba bean meal [10], cottonseed meal, sunflower meal [11], fish and poultry meals, corn gluten, rapeseed meal, sorghum, barley [12], anchovy meal, corn gluten meal, soybean meal, gammaridmeal and crayfish exoskeleton meal [13]. Among the plant protein sources, soybean meal has been used most widely because it has a good amino acid profile, which, as the main source of protein, supports fish growth [14]. Soybeans, however, are not grown widely in Ethiopia; hence there is a need to evaluate soybeans together with other more locally available plant proteins. According to Lovell [15] feed ingredients containing 20% or more crude protein are considered protein sources. In the present study soybean cake (SBC), linseed cake (LSC) and Nigerseed cake (NSC) were selected as dietary protein sources on the basis of their high protein content, availability and use in animal feeds for Ethiopia. Studies conducted on SBC, LSC and NSC showed they have good protein contents (30-40%), depending on processing methods [16,17].

Niger seed is the most important oil crop of Ethiopia, providing 50-60% of the country's indigenous edible oil. It is also minor oil crop in India, Kenya, Uganda, Sudan, Malawi and other African and Indian sub-continent countries. Its seeds are inexpensive to process, and the cake remaining after oil extraction is used as a protein supplement in animal diets [16]. Niger seed cake contains few or no known antinutritional factors [18].

Ethiopia ranks among the top five world producers of linseed and it is the second most important oil crop in the country next to Niger seed [19]. The usefulness of linseed as an ingredient in the diets of fish has been studied by different authors [20-23]. Nutrient and energy

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digestibility studies have been conducted more extensively on soybean for many fish species than on LSC. However, digestibility of NSC in fish diets has not been researched into at all, probably because it is restricted to Eastern Africa, mainly Ethiopia.

This study was conducted to evaluate the apparent digestibility coefficients (ADCs) of dry matter (DM), crude protein (CP) and gross energy (GE) for SBC, LSC and NSC for Nile tilapia, *O. niloticus*.

Materials and Methods

Fingerlings of Nile tilapia of Lake Hora origin with an average weight of 8.9 ± 1.6 g were stocked at 10 fish per tank (60 l tanks) in a water recirculation system established at Ziway Fisheries Resources Research Center by Addis Ababa University Department of Zoological Sciences. All treatments were conducted in triplicate. Fish were fed by hand, twice a day (10:00, 16:00 hours) at a rate of 6% body weight per day. The experiment took 2-3 weeks. The recirculation system was supplied with aerated water from a sump tank thermoregulated at $28 \pm 1^\circ\text{C}$ and a constant photoperiod of 12 hours light/12 hours darkness was maintained. Water quality parameters measured during the experiment averaged (\pm SD): temperature, $28.9 \pm 0.4^\circ\text{C}$; pH, 7.3 ± 0.1 ; ammonia, 0.17 ± 0.08 mg l⁻¹; nitrite, 0.20 ± 0.1 mg l⁻¹; Nitrate, 50 ± 23.2 mg l⁻¹ and dissolved oxygen, 5.4 ± 0.4 mg l⁻¹ and they were within acceptable ranges for tilapia.

Diet Formulation

A reference diet (Table 1) was formulated to satisfy the nutrient requirements of Nile tilapia [24]. It contained 320 g kg⁻¹ crude protein, 100 g kg⁻¹ lipid and 18 kJg⁻¹. The test ingredients for apparent digestibility were soybean cake (SBC), linseed cake (LSC) and Nigerseed cake (NSC). All test feed ingredients were obtained from commercial sources in Ziway, Ethiopia with the exception of soybean cake which was acquired from Addis Ababa oil processing factory outlet.

Three test diets were formulated using 70% reference diet and 30% of each of the test ingredients as described by Cho et al. [25]. This method assumes that there are no interactions among the components of the diet during digestion [26]. Chromic oxide was used as an inert marker at a concentration of 0.5% in the diets. Other supplements used in the diet are indicated in Table 1.

Diet preparation

Fishmeal was processed from waste obtained from a local fish processing plant known as "ZiwayFish Processing Plant". The filleting residues were purchased from the processing plant at a price of 0.15 birr kg⁻¹. The freshly collected filleting residues of tilapia were minced using an electrical meat mincer and then dried in an oven for 48 hours at 75°C. The dried residue was ground into a fine powder using an electrical smashing machine, sieved (0.5 mm mesh size sieve) and then stored in a plastic bag at -18°C in a deep freeze.

The diets were formulated on an as fed basis. Fish meal as the main dietary protein source and wheat and corn grains (milled) as main carbohydrate sources were used in the experiment. A poultry grade vitamin/mineral premix (Table 1) at 50 g kg⁻¹ and a binder (carboxymethyl cellulose, high viscosity) at 20 g kg⁻¹ were added. The vitamin/mineral premix was purchased from the local market in Addis Ababa. This premix is prepared for egg laying hens by an Israeli company called Koffolk Animal Health and Nutrition. Soybean oil was used as the source of lipid in the diets. Chromic oxide was added as an indigestible marker for digestibility study [27].

Diets were prepared by wet extrusion using a meat mincer (Model TJ 22). All ingredients were finely ground and sieved through a 500 µm sieve to obtain a homogenous mixture. The dry ingredients were then weighed out according to the formulation, placed in an aluminum bowl and mixed until uniformly blended using a modified mixer. The resulting homogenate was moistened after addition of water (20%-30%) slowly with continuous stirring until a dough was formed before passing through an electrical meat mincer with a 2.5 mm die. The expelled strands produced from meat mincer were dried in an oven with a convecter fan at 35-40°C for 24 hours. They were then crushed in to crumbles and sieved with 1 mm mesh size sieve. The resulting pellets were packaged in polythene bags and stored in a deep freeze at -18°C. Prepared diet samples were analyzed for proximate composition, energy and chromic oxide.

Faecal collection system

In this study a settling column system was employed for faeces collection, but it was adapted to the 60 l cylindrical tanks used. This collection system employed pipes fitted to the bottom of the rearing tanks with a vertical column and transparent hoses connected to a valve system at the bottom ends, where the faeces were deposited after settling. At the top end of the vertical column an overflow was provided to get rid of excess water flowing through the system. Deposited faeces were collected by opening the valve at the tip end and carefully draining the faeces into centrifuge bottles. The collectors were fixed to the rearing tanks the night before and faeces collected early the next morning. Faeces were immediately centrifuged at 4,300×g for 10 min and the supernatant discarded. Wet settled solids of faeces were frozen at -20°C to retard bacterial decomposition. Faecal samples were later defrosted and oven dried at 60°C, ground and analyzed for crude protein (CP), crude lipid (CL), gross energy (GE) and chromic oxide contents.

Analytical techniques

Ingredients, diets and faeces were analyzed in triplicates for proximate composition according to standard methods [28], and chromic oxide of diets and faeces analyzed by acid digestion with molybdate reagent followed by DPC (Diphenylcarbazine) colorimetry following the procedure in Divakaran et al. [27]. Energy was determined using an Adiabatic Autobomb Calorimeter with benzoic acid as a standard.

The apparent digestibility coefficients (ADC) for the nutrients of the diets were calculated as follows [29]:

Ingredients	Reference diet	Test diets
Test ingredient	-	298.5
Fish waste meal	407.6	285.3
Soybean meal	100	70
Wheat grain	20	14
Corn grain	392.4	274.7
Soybean oil	5.0	3.5
Vitamin mineral premix ¹	50	35
Carboxymethyl cellulose	20	14
Chromic oxide	5.0	5.0

¹Vitamin mineral premix (providing per kg): vitamin A (retinol), 14000 mg; vitamin D₃ (chole-calciferol), 4000 mg; vitamin E (tocopheryl acetate), 10000 I.U; vitamin K₃, 2000 mg; thiamine, 1000 mg; riboflavin, 4000 mg; niacin, 10000 mg; pantothenic acid, 5000 mg; pyridoxine, 750 mg; folic acid, 250 mg; vitamin B12, 8 mg; vitamin H as Biotin, 30 mg; betain, 100000 mg; Antioxidant, 125000 mg. Minerals: Manganese, 80000mg; Zinc, 50000 mg; Iron, 20000 mg; Copper, 5000 mg; Iodine, 1200 mg; Cobalt, 200 mg; Selenium, 200 mg.

Table 1: Composition of reference and test diets (g kg⁻¹) for the digestibility study

$$ADC = 100 \times \left[1 - \left(\frac{F}{D} \right) \times \left(\frac{D_i}{F_i} \right) \right]$$

Where D=% nutrient of diet; F=% nutrient of faeces; Di=% Cr₂O₃ of diet; Fi=% Cr₂O₃ of faeces and ADC of ingredients as,

$$ADC_{test\ ingredient} = ADC_{test\ diet} + \left[(ADC_{test\ diet} - ADC_{ref.\ diet}) \left(\frac{0.7 \times D_{ref}}{0.3 \times D_{ingr}} \right) \right]$$

Where D_{ref} = % nutrient (or kJg⁻¹ gross energy) of reference diet (as fed); D_{ingr} = % nutrient (or kJg⁻¹ gross energy) of test ingredient (as fed).

Digestible protein and energy were calculated as follows:

Digestible protein (DP, gkg⁻¹) = dietary crude protein (gkg⁻¹, dry weight basis) × ADC_{protein}

Digestible energy (DE, kJg⁻¹) = gross energy (kJg⁻¹, dry weight basis) × ADC_{energy}

Statistical analyses in this study were conducted using Minitab Statistical Package (Version 15.0). Differences among dietary treatment means were tested by analysis of variance (ANOVA), and means compared using Tukey's Multiple Comparison Test to test for significance of variation between the means and differences were considered significant at p<0.05. All percentages were arcsine transformed before analysis [30].

Results

Chemical composition and prices of ingredients

Proximate composition and energy contents of the ingredients used in the study are given in Table 2. Crude protein for oilseed cakes ranged from 310 to 393.8 g kg⁻¹ with SBC the highest and LSC the lowest. In contrast, crude lipid was highest for LSC (108.2 g kg⁻¹) and lowest for SBC (74.4 g kg⁻¹). NSC had the highest crude fibre (201.1 g kg⁻¹) level, about three times higher than SBC which had the lowest fibre content (64.8 g kg⁻¹). Gross energy values for ingredients ranged from 17.8 to 21.8 kJ g⁻¹.

The prices of ingredients used in the study are shown in Table 2. Fish meal was the least expensive (0.5 birr kg⁻¹) ingredient as the cost for it is directly converted from the cost of fresh offal (0.15 birr kg⁻¹) and 3.33 kg of offal dried in an oven can make approximately 1 kg of dried fishmeal. SBC and wheat grain were the most expensive ingredients, about double the price (4 birr kg⁻¹) of NSC which was the least expensive among the oilseed cakes.

Chemical composition of test diets

Proximate and energy compositions of the reference and test diets used in the digestibility study are presented in Table 3. Analyzed crude protein, crude lipid, NFE, dry matter, ash and energy contents of test diets showed little variation. However, crude fibre contents of diets varied considerably. Crude fibre of test diets followed similar trend as the test ingredients. Energy contents of the diets ranged between 18.6 and 18.9 kJ g⁻¹.

Nutrient and energy digestibility

Apparent digestibility coefficients (ADCs) of protein, lipid, dry matter and energy in selected test ingredients for Nile tilapia are shown in Table 4. The results indicate that ADCs of the nutrients and energy studied were significantly different between the test ingredients

except for crude lipid digestibility. Generally SBC had the highest ADC coefficients followed by NSC with LSC having the least ADC for energy and nutrients.

Discussion

The suitability of three oilseed by-products (SBC, NSC and LSC) available in Ethiopia were evaluated for their proximate composition and ADC values with the aim of providing information that aids improved formulation of balanced diets for Nile tilapia. The ADC values for dry matter, protein and energy were significantly different between the three test ingredients. However, lipid digestibility was not significantly different between the test ingredients.

In this study the test ingredients used had a high crude protein content and their values were close to previously reported values by Assaminew et al. [17], except for relatively higher crude protein (32.4% Vs 28.1%) and lower crude fibre (25.3% Vs 20.1%) values reported for Niger seed cake in this study. This variation between nutrient compositions of NSC could be due to differences in the origin, state and processing methods used to produce the cakes. The high crude fibre content of Nigerseed cake could limit the inclusion of this ingredient at higher levels in the diets of fish. It has been reported that dietary fibre is not utilized by fish [2].

The digestibility of ingredients provides insight concerning nutrient utilization and should enable better ingredient substitutions in diets designed for target species. The nutrient digestibility will vary depending on the composition of ingredients used [2,8]. The results of this study showed that ADC for dry matter, crude protein and energy in test ingredients were affected by test ingredients. These differences can be explained by the differences in chemical composition, origin and processing of these feed ingredients. The results of the present study indicated that Nile tilapia fingerlings have the capacity to digest protein and lipid satisfactorily in the oilseed by-product ingredients tested.

The overall dry matter digestibility of the test ingredients in the present study ranging from 59% to 78% is in the range reported for

Ingredients	DM	CP	CL	CF	Ash	NFE	GE	Price
Linseed cake	908.8	310	108.2	136.3	82.7	233.4	18.6	7.5
Niger seed cake	928	324.2	92	201.1	90.7	220	18.1	4.0
Soybean cake	938	393.8	74.7	64.8	54	350.7	19.3	9.0
Fish waste meal	950	610.9	187.1	0	220.4	0	21.8	0.5
Wheat grain	875	96	16.54	57.9	13.6	690.9	17.9	8.0
Corn grain	882.9	78.1	42.6	27.1	13.0	722.2	19.0	5.0

*DM (dry matter), CP (crude protein), CL (crude lipid), NFE (nitrogen free extract) and GE (gross energy).

Table 2: Proximate composition (g kg⁻¹ as fed), energy (kJ g⁻¹) and prices (birr kg⁻¹) of individual feed ingredients used in this study.

Components	Reference diet	Test diets*		
		SBC	NSC	LSC
Dry matter	922.8	927.8	924.6	918.9
Crude protein	321.0	341.0	320.3	316.1
Crude lipid	105.8	96.0	101.1	106.0
Crude fibre	18.6	32.2	72.8	53.5
Ash	126.1	98.8	109.8	107.4
NFE	351.5	359.5	320.6	336.0
Chromic oxide	5.2	4.8	4.7	4.9
Gross energy (kJ g ⁻¹)	18.9	18.9	18.6	18.7

*SBC= soybean cake, NSC= Niger seed cake, LSC= linseed cake

Table 3: Proximate composition (g kg⁻¹) and energy of reference and test diets

Components	Soybean	Niger seed	Linseed
Dry matter	78.0 ± 2.6 ^a	70.7 ± 3.0 ^b	59.0 ± 0.5 ^c
Crude protein	87.9 ± 3.2 ^a	72.6 ± 2.0 ^b	62.4 ± 4.2 ^c
Crude lipid	81.4 ± 2.2 ^a	78.9 ± 2.8 ^a	79.4 ± 3.0 ^a
Gross energy (kJ g ⁻¹)	86.0 ± 2.5 ^a	72.9 ± 1.8 ^b	53.7 ± 3.5 ^c
Digestible protein	36.9	25.3	21.3
Digestible energy	17.7	14.2	11.0

Table 4: Apparent digestibility coefficients (%) of protein, lipid, dry matter, energy and digestible protein and energy (g kg⁻¹ and kJ g⁻¹ respectively, dry weight basis) in the test ingredients for Nile tilapia. Coefficients in each row with a different letter are significantly different (P<0.05).

plant protein-rich products (46-86.2%) in the diets of Nile tilapia [8-10]. Dry matter digestibilities in this study were generally lower than those reported for Nile tilapia elsewhere [13]. For example, the lower dry matter ADC of soybean in the present study could be explained by the higher crude fibre content of the product evaluated in this study of 69 g/kg, compared with 39 g/kg in the study by Köprücü and Özdemir [13]. Other studies on fish have also indicated the negative correlation between crude fibre content and dry matter ADC [31-33]. In general, results of dry matter ADC can be used to estimate the amount of solid waste released to the environment and to help determine the environmental impacts of aquaculture production [7,32].

Generally, the protein quality of dietary ingredients is one of the leading factors (apart from palatability) affecting fish performance and protein digestibility (digestible protein) is the first measure of its availability to fish. Protein quality of dietary protein sources depends on the amino acid composition and their digestibility. In the present study, the values obtained for protein digestibility for SBC corroborate previous findings (87.4%-96.2%) for soybean meal in tilapia diets [8,10,12,13,24,34]. Protein digestibility of test ingredients LSC (62.4%) and NSC (72.6%) for Nile tilapia in this study was lower than the reported digestibility coefficients of various other oilseed meals for this species. For example, reported APD in tilapia were 78.5% for cottonseed meal [34], 85% for rapeseed meal [12], 77.6% for peanut meal, 77.8% for canola meal, and 84% for de-gossypoled cottonseed meal [8].

Lower values of LSC protein digestibility in this study could be explained by other dietary factors present in plant protein products such as: i) suboptimal amino acid balance [24]; ii) presence of antinutritional factors [35]; and iii) inadequate levels of energy in linseed meals [36]. Plant products, especially oilseed cakes, usually have poor amino acid profiles and a certain amount of antinutritional factors (ANFs) which could affect nutrient utilization and, consequently, animal growth performance in different degrees depending on the type and amount of the compound [5,20-23]. Although linseed meal has been reported to have one of the best amino acid profiles after soybean meal and the composition fulfills the requirements of amino acid for Nile tilapia, the biological availability of amino acids in linseed to tilapia is less [37]. Linseed contains mucilage (5-8%) which has a large capacity to bind to water and increases intestinal viscosity, thus reducing nutrient digestibility [38]. Major antinutritional factors known to be present in linseed include: cyanogens, phytic acid, tannins, estrogenic factors, antithiamine factor and antipyridoxine factor [5]. For example, the ANF phytic acid has the ability to non-selectively bind to proteins, carbohydrates and minerals (divalent cations such as Ca²⁺, Fe²⁺, Mg²⁺) and inhibit activities of a number of digestive enzymes such as pepsin, trypsin and alpha-amylase [35].

The lower protein digestibility coefficients obtained for the two test ingredients (LSC and NSC) in the present study could not only

be attributed to the ANFs but also to the higher levels of crude fibre that interfere in protein digestion of the diets that contain LSC and NSC. Previous studies [12,31] indicated that feeds with high crude fibre contents have poor nutrient digestibility due to reduced enzymatic access to potential substrates or due to the direct interaction between crude fibre components and the digestive process. Fibre levels as high as 8-12% are tolerated by most fish, but such levels often result in growth depression [39,40]. Fish fed diets high in indigestible fibre increase their feed intake and gastric evacuation time, but the extent to which fish can compensate in this manner is limited [41].

ADC values of fats in fish range from 85% to 95% when administered routinely either alone or in a mixed diet [42]. Reported fat digestibility in other species ranged from 70% to 90% [12,43] and similar values were found for tilapia in this study (78.8-81.3%). The ADCs of energy (53.6%-85.9%) in test ingredients for Nile tilapia in this study are generally in agreement with that reported (39-89%) and (54.8-92.1%) by Sklan et al. [12] and Köprücü and Özdemir [13], respectively. Variation in apparent GE digestibility coefficients of ingredients in this study followed the same trend as that of protein and DM digestibility.

In the present study all three oilseed cakes tested proved valuable as protein sources in the diets of Nile tilapia as indicated by their ADCs. However, best values were observed for SBC. NSC, which was less than half the cost of SBC, appeared to be a good protein feed ingredient for Nile tilapia diets on balance in terms of overall nutrient composition and acceptable digestibility coefficients despite the highest crude fibre content. The LSC generally performed poorly, although it contained 310 g/kg CP. The nutrient and energy digestibilities were very low except for lipid digestibility. It seems that the ANF present in linseed cake may be responsible for the low ADC values. However, further research is required to establish the effect of dietary inclusions of LSC and NSC on productivity and on the various potential methods of increasing their utilization in fish diets before considering these ingredients in production feeds. The results of this digestibility study should contribute towards a better understanding of the nutrition of this species, especially in the grow-out stages.

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