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Protein digestibility status of locally available feed ingredients fed to Nile tilapia (*Oreochromis niloticus*) in the hatchery, Sebeta, Ethiopia

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The nutritional value of a feedstuff isn't only on its chemical composition, however, the content of the nutrients absorb by the aid of the fish. Thus, this study was developed to determine crude protein digestibility status of the five feeds (noug cake (NK), wheat bran (WB), soybean meal (SM), brewery waste (BW), and fish meal (FM) in *Oreochromis niloticus*. The experiment was conducted using 180 *Oreochromis niloticus* population with weight of 28.2 to 28.4 g, distributed in experimental randomized blocks design with five treatments and three replicates per treatment. Digestibility was estimated by the indirect method using chromic oxide at the concentration of 0.50 % of the diet as a marker. The apparent and true digestibility of the protein was estimated for noug cake, 87.1 and 89.1 %; wheat bran 74.3 and 78.7 %; soybean meal 88.3 and 90.0 %; brewery waste 65.4 and 67.8 %; fish meal 85.5 and 87.0 %, respectively in siphoning technique. Similar ($P > 0.05$) results were observed in striping and siphoning methods in all experimental ingredients. Soybean, noug cake and fish meal had better ($P < 0.05$) crude protein digestible by *Oreochromis niloticus* than wheat bran and brewery waste. Brewery waste was the lowest digestible by *O. niloticus*. The growth performance of the fish also in line with the crude protein digestibility status of the ingredient hence, the lowest growth performance was recorded in fish consumed brewery waste. This indicates, brewery waste isn't well digestible by *O. niloticus*.

Key words: Siphoning, striping, ingredients, digestibility, *Oreochromis niloticus.*

INTRODUCTION

The nutritional value of a feed is not exclusively based on its chemical composition but also on the amount of nutrients that can be absorbed by the fish (Ibrahim, 2005; Patrícia, 2012). Digestibility describes the fraction of the nutrient in the ingested feedstuff that is not excreted in the faeces (Cho and Kaushik, 1990; Allan et al., 2000). For the duration of fish feed system, the nutrient compositions of the feeds and the biological utilization of the nutrients in every of the inclusion ingredients for the species should be considered (Maina et al., 2002; National Research Council, 2011). Studies on nutritional requirements and evaluation of feed for the specific fish species have prioritized the test of protein, which are the most highly-priced element in feed formulation (Felipe et

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al., 2011). The protein quality and quantity is the basic factor influencing fish growth performance and digestibility status of the feed (Yu et al., 2013). Hence, the digestibility of each ingredient in fish feed formulation is the basic aspect in evaluating the suitability of feeds for the growth of the fish.

There are two types of digestibility studies, true and apparent digestibility. True digestibility is the differences between ingested nutrient and egested nutrient by considering the endogenous losses but apparent is only the difference (Sakomura and Rostagno, 2007; Felipe et al., 2011). There are two strategies which determine the digestibility coefficients of the feed stuffs. These are direct and an indirect measurement of the amount of nutrient ingested and subsequently excreted, but the indirect method is the preferred technique to determine the fish digestibility coefficient (Sakomura and Rostagno, 2007). This method involves the use of a non-digestible marker. A dietary marker is a substance that is not metabolized by the animal but used to determine the proportion of feed digested and excreted as waste (Merchen, 1988). Dietary markers must have no toxic effects, cannot be lost in digestion, cannot add considerable mass, and must be easily and equally mixed into feed. There are different types of dietary markers like ash, titanium dioxide (TiO_2) and chromium oxide (Cr_2O_3). Most of the time chromic oxide was used in digestion trials with sheep, horses, cattle, pigs, fish, and humans (Raymond and Minson, 1955; Rahman et al., 2016). It is assumed that the quantity of the markers within the feed and faeces remains consistent throughout the experimental period and that every one of the ingested markers will appear within the faeces. The digestibility of the nutrient in question can be decided by way of assessing the difference between the feed and faecal concentrations of the markers and the nutrient. The indirect method has the advantages that it eliminates the need to quantitatively collect all of the excreta, and the test fish can consume voluntarily (Ibrahim, 2005).

Proper faecal collection to prevent loss of soluble nutrients into the water is vital in digestion trials, whether or not the direct or indirect method is used (Felipe et al., 2011; Rahman et al., 2016). Data from Smith et al. (1980) imply that a significant amount of the fecal nitrogen from rainbow trout is in liquid form and may leach out of the faeces into the water prior to collection. The calculation of digestibility, when a high amount of soluble nutrients have leached from the faeces, gives digestion coefficients that are erroneously high (Smith and Lovell, 1971; Windell et al., 1978). Some investigators have consequently chosen to collect the faeces directly from the rectum using anal aspiration (Windell et al., 1978), surgical excision (Smith and Lovell, 1971), or stripping (Nose, 1960) to minimize the problem of nutrient leaching. Austreng (1978) suggested that if stripping was used to remove the faeces from the rectum, care should be taken not to obtain partially digested feed or biological fluids from the gut. Choubert (1979) and Cho and Slinger (1979) have shown that, that if excretions are removed from the fish tank soon after expulsion, the collection of passively excreted faeces can give good digestibility information.

In Ethiopia, there are diverse agricultural and agro-industrial by-products which are not consumed by humans and have higher potential for feed sources of fish (Kassahun et al., 2012). But the digestibility of locally available fish feed sources is not studied so far. This study was therefore proposed to assess the crude protein digestibility of some locally fish feed ingredients (Noug cake, wheat bran, brewery waste, fish meal and soybean) by *Oreochromis niloticus* in the hatchery.

MATERIALS AND METHODS

Study site

The present experiment was carried out at the National Fisheries and Aquatic Life Research Center (NFALRC) hatchery from August 2018 to October 2018 which is located some 24 km Southwest of Addis Ababa (8°55.076'N; 38°38.161'E) at an altitude of 2240 m a.s.l.

Feed ingredients and preparation

The locally available fish feed ingredients: wheat bran, noug cake and starch were purchased from Addis Ababa, brewery waste was collected from meta brewery factory, fish meal from Bahir Dar and soybean was available in NFALRC feed store. Before used as a feed, soybean was processed through boiling and roasting manually to reduce the oil content and anti-nutritional effects or chemicals. Chromium oxide was imported from Austria and used as an external marker; it was incorporated into the experimental ingredients at 0.5% used as an indicator for determining the digestibility (Felipe et al., 2011; Rahman et al., 2016). All chemical analysis of feed ingredients and faeces was carried out as described in AOAC (1990) in triplicates and in JIJE Analytical *Testing* Service Laboratory (Doc. No: JATSL/F5.10-3) (Table 1).

Fish and experimental condition

A total number of 180 *O. niloticus* with average initial weight of 28.1-28.4 g were used and stocked into18 experimental aquaria with 100 liter water holding capacity designed in a recirculating system with partial water renewal. The stocking density was 10 fish per aquarium in triplicates designed at completely randomized design (CRD) for 90 days. Each aquarium was clean, well-prepared, installed and filled with water. This system was composed of a biological filter, comprising three tanks of 200 liter capacity each and filled with gravel, and a sand filter. Fish were stocked seven days prior to the start of the faecal collection period to allow for adaptation to the experimental conditions. During this period, fish were fed the maintenance diet. Afterwards, each diet was randomly assigned to the aquaria.

Faeces collection and digestibility assessment

The first 7 days of each period were used for adaptation to the feed and no faeces was collected. This time period was considered

Table 1. Experimental diets and their proximate composition on dry matter basis.

ingredients	Ingredients inclusion level (%)					
	Protein free diet	Noug cake	Wheat bran	Soybean meal	Brewery waste	Fish meal
Noug cake (NK)		91.5				
wheat bran (WB)			91.5			
Soybean (SB)				91.5		
Brewery waste (BW)					91.5	
Fish meal (FM)						91.5
Corn starch	91.5					
Soybean oil	6	6	6	6	6	6
Premix	2	2	2	2	2	2
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (g 100 g^{-1})						
Dry matter (%)		90.8	88.9	96.2	88.8	92.3
Protein (%)	0.00	32.62	14.95	37.39	26.92	45.97
Ash (%)		10.4	3.4	6	10.2	20.4
Fat (%)	0.00	7.2	4.9	8.1	5.2	12.7
Crude fibre (%)	3.90	10.5	11.4	8.1	11	0.2

sufficient for the fish to achieve complete evacuation of previous diets and metabolically adapt to the new diet. Fish were then fed the experimental diets 5 % their body weight twice per day at 09:00 and 16:00 h. Each day, 30 min after the second feeding, the aquaria were cleaned and 50% of water renovated to remove any residual particulate matter such as faeces and uneaten feed. After 5 h, the faeces which settled in the bottom of each aquarium was collected and some of the faeces was collected by striping five fish from each replicate after the fish were anaesthetized with a 0.2 gL^{-1} suspension of MS-222 (Maina et al., 2002; Felipe et al., 2011; Rahman et al., 2016) for every week. Faeces was then centrifuged at 5000 × for 10 min and the supernatant discarded (Felipe et al., 2011). Faeces was then dried at 105°C and stored at deep freeze until analysis. The faeces collection continued throughout the experimental period and faeces from each aquarium was pooled.

Chromic oxide was determined in the diets and faeces according to Furukawa and Tsukahara (1966). For each replicate, 200 mg of sample was weighed and transferred to the digestion tubes and this occurred in two phases: first, 5 ml concentrated nitric acid (HNO_3) was added to the digestion tubes and the tubes placed in a heating mantle for approximately 30 min for solubilization of all organic material. Afterwards, 3 ml of sulfuric acid (H_2SO_4) was added to the digestion tubes that were put back in the heating mantle for 30 min. When cooled to ambient temperature, 10 ml of deionized water was added to the tubes. The concentration of chromic oxide was then measured at 350 nm in a UV spectrophotometer. According to Furukawa and Tsukahara (1966), the concentration of chromic oxide was calculated as follows:

(1) The amount of chromic oxide (mg) present in the sample.

$$X = \frac{1}{4} * \frac{Y - 0.0032}{0.2089}$$

Where: Y=absorption, 0.0032 & 0.2089 are constants.
X= chromic oxide (mg)

(2) To calculate the % of chromic oxide (C. O %) $C.O.\% = 100\frac{X}{A}$

Where: X=weight of chromic oxide, A=weight of sample

Apparent and true digestibility of protein was determined by calculating the indigestibility factor described by Rostagno and Featherston (1977), as follows,

Indigestibility factor (IF) = $\frac{Cr_2O_3 diet}{Cr_2O_3 feces}$

(3) Apparent crude protein digestibility coefficient (ApCPDC)

ApCPDC % = $\frac{Dietcp - (cpE1 * IF1) * 100}{Diet\ cp}$

E1= faeces from tested diet

(4) Crude protein true digestibility coefficient (CPtDC)

CPtDC % = $\frac{Dietcp - (cpfeces * IF1 - cpe * IF2) * 100}{Diet\ cp}$

Where: cpe = endogenous crude protein excreted in the faeces, IF1= indigestible factor for the tested diet and IF2=indigestible factor for the protein free diet (PFD)

Water quality analysis

The water temperature was controlled by thermostat and ranged from 26 - 28°C throughout the culturing period. Continuous flow of recirculating water was allowed for 24 h per day; 2 litters per minute flow was rare except faeces sampling period (5 to 7 h per week). Water quality parameters (Temperature and dissolved) were measured in every three days and total ammonia nitrogen level was measured once every two weeks. Total ammonia nitrogen (TAN) was determined by using the Indo-Phenol blue method (Khosravi et al., 2012).

Statistical analyses

Data are presented as mean ± standard Error. All data were subjected by one-way analysis of variance (ANOVA) using a significance level of $P < 0.05$. Tukey's multiple range test was applied to determine differences among means. All statistical analyses were carried out using the IBM SPSS version 20.0.

RESULTS AND DISCUSSION

The apparent and true digestibility of protein for the five ingredients is shown in Table 2. The protein quality of the feed ingredients has been always the basic aspect for the growth of fish (Yu et al., 2013). The digestibility of protein in both techniques (siphoning and striping) revealed that fish meal (FM), soybean (SB) and noug cake (NK) had better ($P < 0.05$) crude protein digestible via *O.niloticus* ($P < 0.05$) than wheat bran (WB) and brewery waste (BW), and brewery waste was the lowest protein digestible ingredient. This suggests that each of these feed ingredients has a specific crude protein utilization efficiency for *O. niloticus*. Similar ($P > 0.05$) results were observed in the two faeces extraction strategies in this study.

The crude protein digestibility of soybean meal was lower than Sklan et al. (2004) and Felipe et al. (2011) which are 91.12 and 96.2% respectively. This was probably the anti-nutritional factors (trypsin inhibitors and phytic acid) (Webster et al., 1992). In this study, the amount of oil content and anti-nutritional chemicals were tried to reduce through roasting and boiling prior to use as feed. However, the ant-nutritional chemicals are removed with oil and deactivated well throughout boiling and roasting using extraction and roasting machines (Webster et al., 1992; Allan and Booth, 2004). But the above machines are not available in the center and we used manual methods which are most practicing in Ethiopia for soybean ingredients used as a fish feed. Hence, before using soybean as a fish feed, the oil and anti-nutritional effect (chemical compounds) must be removed and destroyed at a required level (Maina et al., 2002) to increase its acceptability, digestibility status and reduce the anti-nutritional and health effects of the fish species. On the other hand, extruded soybean feed is the best to improve nutrient utilization and destroy some anti-nutritional factors (Robinson et al., 2001; Barrows et al., 2007).

Fish meal crude protein digestibility is better than Felipe et al. (2011) which is 83% % and lower than stated through Muhammad et al. (2004), 88.8%. The protein digestibility status of fish meal on *O. niloticus* is shown to have variations in many studies due to different reasons. For instance, Romero et al. (1994) reported 84.5 to 97% of digestibility from 27 samples and Rahman et al. (2016) stated 87 to 95%. The variations between the digestibility of fish meal in this research and in other studies used for comparison can be explained by several factors, such as the inclusion level, experimental condition, processing of ingredients and feeds, cultured fish species type, raw materials, location, equipment type and the method used to collect faeces (Romero et al., 1994; Aksnes et al., 1997; Maina et al., 2002; Rahman et al., 2016).

The crude protein digestibility status of noug cake is among the better digestible group ingredients; however, there is no published information related to noug cake digestibility. Consequently, this report is the first because as far as we know noug (*Guizotia abyssinica*) is a semi domesticated oilseed crop, which is primarily cultivated in Ethiopia (Dempewolf et al., 2015). The apparent and true digestibility of wheat bran is similar to that of Maina et al. (2002) and lower than Felipe et al. (2011), which are 75.0, 78.21 and 88.8%, respectively. This is due to the difference of the milling process, seed variety and the soil types where the ingredients were grown (Degani et al., 1997). Brewery waste was the lowest in this study and information on the digestibility of protein in brewery waste for *O. niloticus* is limited. The lowest digestibility of brewery waste is because of excessive fibre (indigestible material) and low nutrient contents present in the feed (Li et al., 2013). Many studies have shown that the crude fibre content alters the digestibility and digestive tract morphology of the fish (Buhler and Halver, 1961; Leary and Lovell, 1975) and they reported the levels above 5.0% of crude fibre influenced the digestibility of crude protein in Nile tilapia. The fibre contents of the experimental ingredients ranged from 8.1 to 11.4% except fish meal which was 0.2% (Table 1).

The ash contents of the ingredients also influenced on the digestibility of the feed (Felipe et al., 2011; Rahman et al., 2016). The lowest dry matter of the brewery waste may be attributed to the high ash content and resulted in decreased digestibility value. In this study, fish meal had the highest ash content component (20.4%) and it is similar to other studies (Zhou and Yue, 2012; Rahman et al., 2016); this indicates the lower quality of the fish meal. In line with Rychly and Spannhof (1979), if the protein digestion coefficients for the ingredients are between 75 to 95 %, the ingredients must be incorporated during feed formulation. Therefore, in the present study, the protein digestibility of the experimental feeds was above 75% except brewery waste. The main differences between the true and apparent digestibility status of the tested ingredients were due to the presence of endogenous losses (intestinal cells, enzymes and microbial matter) in the fish body and they were measured by using protein free diets (Sakomura and Rostagno, 2007; Felipe et al., 2011) as described in the methodology part (Table 1).

The effect of dietary protein level on the growth performance of *O. niloticus* is shown in Table 2. At the start of the study, fish in all groups had similar mean weight ($P > 0.05$). After 90 days, the *O. niloticus* growth

Table 2. Means (± SE) of apparent and true protein digestibility coefficient (%) of the tested ingredients in siphoning and striping techniques.

Experimental diets	Siphoning, ACPDC%	Strip ACPDC%	Siphoning, CPtDC %	Strip CPtDC %	Final weight (g)	Weight gain (g fish^{-1})	FCR
Wheat bran	74.3±0.2[a]	75.1±0.2[a]	78.7±0.2[ab]	79.4±0.2 [ab]	31.1±0.3[a]	2.7	3.8
Noug cake	87.1±0.3[b]	87.4±0.2[b]	89.1±0.3[ac]	89.4±0.2 [ac]	35.2±0.1[b]	7	3.4
Soybean meal	88.3±0.1[b]	88.6±0.3[b]	90.0±0.1[ac]	90.3±0.3 [ac]	36.3±0.4[b]	8.1	3.3
Fish meal	85.5±0.1[b]	85.9±0.2[b]	87.0±0.1[ac]	87.3±0.2 [ac]	37.6±0.7[b]	9.4	3.3
Brewery waste	65.4±0.1[c]	66.3±0.2[c]	67.8±0.1[bc]	68.8±0.2[bc]	30.7±0.5[c]	2.6	4.2

Similar letters across the rows and columns refer to non-significant differences and different letters represent significant differences.

rate was relatively better fed on diets (FM, SB and NK) than the ones consumed diets (WB and BW). Fish that consumed the brewery waste had the lowest growth performance and digestible value. The minimum and maximum temperature and dissolved oxygen of the experimental units ranged from 26 - 28°C and 4.4 - 7.2 mgL^{-1} respectively and relatively within the range of the optimal requirement for the *O. niloticus* feed and growth (Wetzel, 2001; Halver and Hardy, 2002; Azaza et al., 2008). However, the growth performance of the fish was relatively poor as compared to other studies (Maina et al., 2002; Liti et al., 2005; Adamneh et al., 2007; Zenebe et al., 2012). This is because in this study, the fish received only single protein sources (ingredients) but the other studies incorporated reference diets (~ 70%) with the experimental components (Maina et al., 2002; Felipe et al., 2011); but reference diet is not available in Ethiopia. Zenebe et al. (2012) used single protein sources (NK, WB and BW) and found better growth performance compared to the present study. This is because the experiment has been executed in ponds and thus, availability of natural planktons unlike indoor experimental fish only depends on artificial diets.

Generally, the apparent and true digestibility status of protein in this study revealed that the protein of soybean (SB), fishmeal (FM) and noug cake (NK) is relatively better accepted by *O. niloticus* compared to other tested diets. But their effects on the growth performance of the fish were not significant in comparison to other studies. This could be attributed to numerous factors, such as the inclusion level of the ingredients, possible anti-nutritional factors, and the technique used to collect faeces, the method and processing of feeds and ingredients and even the soil types where the crops were grown.

CONCLUSION AND RECOMMENDATION

Due to the digestibility status and their impact on the growth performance of the *O. niloticus*, most of the experimental ingredients were above 75 % digested through the fish and this indicates it is possible to incorporate in *O. niloticus* feed formulation. SB and NK and FM protein digestibility was selected ingredient for *O. niloticus* diets formulation in terms of overall digestibility status. BW has the lowest protein digestible in this study and is needed to remove these ingredients for *O. niloticus* feed formulation and it has high contents of ash and crude fibre. Therefore, this leads to increased waste production and reduces water quality (Maina et al., 2002; Rahman et al., 2016). The use of true digestibility values of the ingredients tested, bearing in mind the differences found for the apparent digestibility values, will allow more appropriate and accurate diet formulation for Nile tilapia, which can improve animal performance. According to the present study, the siphoning and striping faeces extraction techniques show similar results and are used better alternatively.

To our knowledge, this is the first report on protein digestibility status determined for *O. niloticus* in Ethiopia. This information provides an important basis for further investigation of single or mixed ingredients diet preparation or substitutions for evaluation of nutrients requirements of *O. niloticus*, in order to maximize performance and to minimize production costs and waste production. The authors also recommend to give more attention to the sources and quality of the selected ingredients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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