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Apparent Digestibility Coefficients of Protein in Selected Feedstuffs for Juvenile Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758)

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Abstract: Apparent Digestibility Coefficients (ADC) of protein in anchovy (*Engraulis encrasicolus*), corn (*Zea mays*) gluten, soybean (*Glycine hispida*), gammarid (*Gammarus kischineffensis*) and crayfish (*Astacus leptodactylus leptodactylus*) exoskeleton meals was determined for juvenile Nile tilapia, *Oreochromis niloticus*. In each of five trials, a reference diet was mixed with test ingredients in a 70:30 ratio to produce a series of test diets. Chromic oxide was added as a marker to the reference diet. The ADC for protein were; anchovy (90.2%), corn gluten (88.5%), soybean (87.6%), gammarid (76.3%) and crayfish exoskeleton (70.9%) meals ($p<0.05$ for each case, except for anchovy, corn gluten and soybean meals). The results of the study indicated that *O. niloticus* may efficiently utilise gammarid and crayfish exoskeleton meals as dietary protein source, like anchovy, corn gluten and soybean meals.

Key words: Nile tilapia, *Oreochromis niloticus*, apparent protein digestibility, feedstuffs

INTRODUCTION

In recent years, the intensification of tilapia fry production in Turkey has made it essential to develop complete and supplemental diets for use in hatcheries and nursery ponds. Traditionally, fish meal has been the main source of protein in diets for fish fry. However, the increasing cost of fish meal has restricted its use as a protein source for fry diets. Therefore, it is a matter of urgency that suitable alternative protein sources for tilapia fry diets be found. An effective feed can be prepared when information on digestibility of nutrients in feedstuffs has been considered in its formulation

For tilapia diets typical plant protein alternatives have included soybean, sunflower seed, corn, alfalfa, cottonseed, poultry offal, lupin seed, maize, wheat, various cereals, brewers grain, cocoa husk meals, etc.[1-6]. In addition to these, the gammarid and crayfish (crustacean) exoskeleton meals have high nutrition values. Especially essential amino acid content of gammarid is sufficient to meet the dietary requirements of fish[7]. It was also suggested[8] that the gammarid could be fed live to fish in cages and ponds, or that they could be processed into a feed pellet. These crustacea are significantly amount harvested from many lakes and rivers in Turkey and used in commercial fish feeds[9]. Total crustacea production in the world and Turkey was about 934.769 and 21.867 tons in 1995, respectively[10].

Aim of the present study was to investigate Apparent Digestibility Coefficients (ADC) of protein in the anchovy, corn gluten, soybean, gammarid and crayfish exoskeleton meals as protein sources for juvenile Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

This study was performed at the aquarium fish reproduction unit of the Aquaculture Faculty of Firat University, Elazig, Turkey. Each treatment had three replicates, 15 juvenile Nile tilapia (*O. niloticus*) per replicate, with mean initial fish weights of 13.8±0.1 g. Each treatment was randomly assigned to 3 glass aquaria (25 L). Water temperature in the aquaria was maintained at 27±1 °C and the fish were subjected to a 12 h photoperiod using fluorescent lights. Supplemental aeration was provided to maintain dissolved oxygen levels near saturation. The water was constantly replaced in aquaria by continuous flow at a rate of 250 mL/min. Duration of the feeding trial was 30 days.

The apparent digestibility coefficients of five protein ingredients, obtained from a local supplier, was determined. The feedstuffs consisted of anchovy (*Engraulis encrasicolus*) meal (mechanical extracted), corn (*Zea mays*) gluten meal (dehydrated), soybean (*Glycine hispida*) meal (solvent extracted), gammarid (*Gammarus kischineffensis* Schellenberg, 1937) meal

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Table 1: Composition of the reference and test diets (%)

Ingredients	Reference diet[I]	Test diets
Anchovy meal	15.00	
Soybean meal (solvent extracted)	43.00	
Wheat flour	38.70	
Sunflower oil	1.90	
Antioxidant [a]	0.10	
Vitamin premix [b]	0.25	
Mineral premix [c]	0.05	
Chromic oxide (Cr_2O_3)	1.00	
Reference diet		70
Feedstuff		30
Total	100.00	100

[I]Reference diet formulation based on Lovell[11]
(a) Antioxidant (mg kg^{-1} dry diet): butylated hydroxytoluene 12.5
(b) Vitamin premix (IU or mg kg^{-1} dry diet): retinol 4000 IU, calciferol 2000 IU, tocopherol 50, menadione 10, thiamin 10, riboflavin 15, niacin 30, pantothenic acid 50, pyridoxine 10, cobalamin 0.02, folic acid 5, biotin 1, choline chloride 500, ascorbic acid 200
© Mineral premix (mg kg^{-1} dry diet): manganese 25, iron 44, zinc 100, copper 3, iodine 5, selenium 0.3

(cooked, 65°C; ground, 0.2 mm in size) and crayfish (*Astacus leptodactylus leptodactylus* Eschscholz, 1823) exoskeleton meal (cooked, 65°C; ground, 0.2 mm in size). In each of the digestibility trials (Table 1) a reference diet[11] was mixed with each of the test ingredients at a 70:30 ratio (as is basis[12] to produce a series of test diets (test diets no; 1, 2, 3, 4 and 5, respectively). Chromic oxide was included in the reference diet as a marker. Table 2 show the chemical composition of the feedstuffs and experimental diets.

Ground ingredients for each diet and marker were mixed thoroughly. Cold water was added to the mixture and stirred thoroughly to form a thick paste. This was then pelleted by extruding the paste through a mincer, resulting in "spaghetti-like" strings. They were placed on aluminum foil and dried in air current at room temperature for 24 h. The feeds were then broken up and sieved into convenient pellet sizes (3-5 mm) for the fish and stored in polythene bags at 4°C until used. The amount of diet needed weekly was then kept at room temperature[4,12].

The fish were acclimated to the experimental system for 7 days before the start of the experiment. Faeces collection started 4 days after changing to the experimental diets to allow evacuation of all previously ingested material. Faecal samples were collected using the faeces settling column (Guelph System) described by Cho *et al.*[13]. The velocity of the water flow was adjusted to minimize settling of the faeces in the drainpipe and maximize recovery of the faeces in the settling column. The experimental diets were each randomly allocated to a collection unit.

During the trial, fish were hand-fed to near-satiation three times per day between 0900 and 1600 h. One hour after the last daily meal, the drainpipe and the settling column were brushed out to remove feed residues and faeces from the system. One-third of the water in the aquaria was drained to ensure that the cleaning procedure was complete. At 08:30 h following day, the settled faeces and surrounding water were gently withdrawn from the base of the settling column into a centrifuge bottle. These faeces were free of uneaten feed particles and considered to be a representative sample of the faeces produced throughout the 24 h period.

Daily faeces collected were centrifuged at 5000 X g for 15 min and the supernatant discarded. The faeces were then freeze-dried, ground using a 1 mm screen and stored at -35°C until required for analysis[14].

Crude protein, fat, ash, fiber, moisture and gross energy were determined for the feeds according to AOAC[15] methods. Crude protein content was also determined for faecal samples from each aquarium. Marker content of diets and faeces was determined in duplicate following Furukawa and Tsukahara[16]. The ADC% of protein for the test and reference diets were calculated as follows[17]:

$$ADC = 100 \times [1 - (F/D) \times (Di/Fi)]$$
$$ADC_I = [ADC_T - (0.7 \times ADC_R)]/ 0.3$$

where:

D	=	% protein of diet,
F	=	% protein of faeces,
Di	=	% marker (Cr_2O_3) in diet,
Fi	=	% marker (Cr_2O_3) in faeces,
ADC_T	=	% apparent digestibility coefficient of test diet,
ADC_R	=	apparent digestibility coefficient of the reference diet,
I	=	ingredient under investigation.

Data were subjected to one-way ANOVA. When appropriate, the Duncan's New Multiple Range test was applied to evaluate difference between means.

RESULTS AND DISCUSSION

The ADC of protein in the anchovy, corn gluten, soybean, gammarid and crayfish exoskeleton meals for juvenile Nile tilapia (*Oreochromis niloticus*) was determined. The highest ADC of protein was obtained from anchovy meal (90.2%), corn gluten meal (88.5%) and soybean meal (87.6%), respectively (Table 3). The ADC of protein significantly decreased with gammarid meal (76.3%) and crayfish exoskeleton meal (70.9%) ($p<0.05$, for each case, except for anchovy, corn gluten and soybean meals).

Table 2: Chemical composition of the reference diet, feedstuffs and test diets (in parentheses)

	Component (%, dry matter basis)						
Feedstuffs and diets	Crude protein	Crude fat	Fiber	Ash	NFE[1]	Gross energy (kcal g^{-1})	Moisture
Reference diet	35	11	11.6	4.8	37.6	3.1	8.2
Anchovy meal	71.0 (46.9)	8.5 (10.2)	1.2 (8.1)	14.1 (7.9)	5.2 (26.9)	4.6 (3.6)	8.0 (8.1)
Corn gluten meal	66.4 (45.2)	2.0 (8.0)	1.7 (8.3)	2.3 (4.0)	27.6 (34.5)	3.9 (3.4)	8.7 (8.4)
Soybean meal	53.9 (41.4)	1.0 (7.7)	3.8 (9.0)	6.4 (5.3)	34.9 (36.6)	3.5 (3.2)	9.1 (8.5)
Gammarid meal	44.3 (38.3)	11.8 (11.3)	6.8 (9.9)	27.8 (12.5)	9.3 (28.0)	3.3 (3.2)	9.0 (8.5)
Crayfish exoskeleton meal	40.3 (36.7)	6.8 (9.6)	10.5 (11.2)	30.3 (13.3)	12.1 (29.2)	2.7 (3.0)	9.4 (8.6)

[1]Nitrogen-free extract (NFE) calculated by difference

Table 3: The ADC of protein for reference diet and feedstuffs in digestibility trials for *O. niloticus*

Reference diet and feedstuffs	ADC of protein[1] (%)
Reference diet	86.9±1.0
Anchovy meal	90.2±1.3[a]
Corn gluten meal	88.5±2.5[a]
Soybean meal	87.6±1.1[a]
Gammarid meal	76.3±0.4[b]
Crayfish exoskeleton meal	70.9±1.2[c]

[1]Values are means±SEM for n = 3 replicate. Means with the different superscript within the same column are significantly different ($p<0.05$)

The differences in protein digestibility may explain by differences in chemical composition, origin and processing of these feedstuffs (Table 2). The high contents of ash in crayfish exoskeleton meal (30.3%) and gammarid meal (27.8%), nitrogen-free extract in soybean meal (34.9%) and corn gluten meal (27.6%) may affect on protein digestibility of these ingredients.

The ADC of protein in these feedstuffs for *O. niloticus* are higher or lower than reported the same values for some feed ingredients. For example, sesame (78.9%), mustard (85.3%) and linseed meals (85.8%) in *Cyprinus carpio*[18], corn (83-84%), wheat (75-89.6%), soybean (91-94.4%) and fish meals (84.8-86%) in *O. niloticus*[2,19], alfalfa (66%), corn (84%), wheat (90%), soybean (94%) and fish (menhaden) meals (85%) in blue tilapia[3], gammarid (67.9-76%) in *Oncorhynchus mykiss*[20], soybean (96.2%) and fish meals (90.8%) in *Ctenopharyngodon idella*[21]. Part of the variability in ADC of protein may explain by differences in chemical composition, origin and processing of these various feed ingredients, methods of faeces collection and fish species.

There are many factors that can affect digestibility work in aquaculture species, but the acceptability of results in this study rested on the recommended procedures that were followed. In conclusion, from the digestibility viewpoint it appears that *O. niloticus* may efficiently utilise gammarid and crayfish exoskeleton meals as dietary protein source, like anchovy, corn gluten and soybean meals. However, further studies would be required to improve the digestibility in order to derive optimum benefits of gammarid and crayfish exoskeleton meals in fish feeding.

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