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Protein Digestibility Studies in *Oreochromis niloticus* Using Chromic Oxide Indicator

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Abstract

The indicator method, 0.5% or 1.0% chromic oxide, and the dissection technique of collecting feces, were used to evaluate the digestibility of protein by *Oreochromis niloticus*. High and low protein feedstuffs were evaluated for true and apparent digestibility coefficients in isocaloric diets formulated to contain 30% and 15% protein, respectively. The stomach and subsequent segments of the intestine were analyzed for their protein and chromic oxide content.

Results indicated that animal and plant proteins were highly available to *O. niloticus*. The true digestibility coefficients of tested feedstuffs were: corn gluten meal 97%; soybean meal 93%; fish meal 92%; shrimp 87%; rice bran 93%; azolla 75%; and copra meal 56%. The nonprotein diet showed that the anterior intestine was the major site of metabolic nitrogen excretion. The major site for nitrogen absorption in the intestine varied with different feedstuffs. The similar true digestibility coefficients of casein in the 15% and 30% protein diets indicated that true protein digestion was independent of dietary protein level.

The effects of fat, carbohydrate and energy levels on protein digestibility were determined in a 2 x 2 factorial experiment using two levels of energy (2,800 kcal.kg^{-1} and 3,800 kcal.kg^{-1}) and two levels of fat (6% and 12%), at 30% protein. Increase in fat level from 6% to 12% had no effect on protein digestibility at 2,800 kcal.kg^{-1} energy level. High energy diets giving protein:energy ratios below 83 mg.kcal^{-1} yielded significantly ($P \leq 0.05$) lower protein digestibility. The results suggest that dietary fat up to 12% provides energy to *O. niloticus* to spare protein as an energy source without negatively affecting protein digestibility.

Introduction

Protein is the most expensive component in artificial diets for fish. Yet, fish diets contain high levels of protein which is used by the fish to produce both body protein and energy (National Research Council 1983). An approach to lower the cost of fish feeds is to "spare"

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protein as an energy source with less expensive sources like fats and carbohydrates. Dietary protein is efficiently used by increasing dietary lipid and/or carbohydrate provided that protein intake is above the critical threshold required by particular fish (Atherton and Aitken 1970; Lee and Putnam 1973; Piefer and Pfeffer 1980; Beamish and Thomas 1984; Kaushik and De Oliva Teves 1985; Beamish and Medland 1986). However, high levels of carbohydrate have been shown to decrease protein digestibility (Inaba et al. 1963; Kitamikado et al. 1964; Page and Andrews 1973; Austreng et al. 1977).

Another approach to cut feed costs is to substitute expensive feedstuffs from animal sources like fish meal for less expensive plant sources (Jackson et al. 1982; Jauncey 1982). This approach requires the evaluation of protein availability of feedstuffs to the fish either through feeding trials, which are dependable but slow and expensive, or digestion trials which are cheaper and require shorter time to conduct. In the latter method, true protein digestibility appears to be independent of dietary protein level unlike apparent protein digestibility (Inaba et al. 1963; Nose 1967; Ogino and Chen 1973; Page and Andrews 1973).

In this study, apparent and true digestibility of protein by *O. niloticus* in various feedstuffs were evaluated in diets that contained either 30% or 15% protein and about the same level of carbohydrate and fat. The effects of dietary fat, carbohydrate and energy levels on protein digestibility in diets were also determined.

Materials and Methods

Protein digestibility of various feedstuffs was determined in isocaloric and isonitrogenous diets. In Experiment 1, four feedstuffs from animal and plant sources containing relatively high protein levels were used as protein sources in diets that contained 30% crude protein (Table 1). In Experiment 2, feedstuffs from plant sources containing low levels of protein were evaluated for protein digestibility in 15% protein diets (Table 2). The digestibility of casein was also determined at two protein levels. The diets used were formulated to provide all the nutrient needs of the fish. Experiment 3 was designed to determine whether the digestibility of protein was influenced by the level of fat or energy in the diet. The digestibility of protein from fish meal was determined in diets (Table 3) containing two levels of fat (6% and 12%) and two levels of energy (2,800

Table 1. Composition of the protein-free diet and the experimental diets prepared at 30% crude protein level (Experiment 1).

Ingredients	Diets 1	2	3	4	5	6
Casein	-	33.3	-	-	-	-
Shrimp meal	-	-	51.1	-	-	-
Fish meal	-	-	-	46.6	-	-
Soybean meal	-	-	-	-	66.5	-
Corn gluten meal	-	-	-	-	-	50.4
Dextrin	60	30	24.5	25.4	3.0	16.4
Vegetable oil	10	10	8.4	5.7	9.0	9.4
Vitamin-mineral mix[a]	6	6	6	6	6	6
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5
Carboxymethyl cellulose	3	3	3	3	3	3
Celite (filler)	20.5	17.2	6.5	12.8	12.0	14.3
Total	100	100	100	100	100	100
Calculated composition (dry basis):						
% Crude protein	0	30	30	30	30	30
% Crude fat	10	10	10	10	10	10
% Nitrogen-free extract	60	30	30	30	30	30
% Crude fiber	20.5	17.2	10.5	13.1	16.3	15.6
% Ash	4	4	14.1	11.4	8.2	8.9
ME (kcal.kg^{-1})[b]	3,300	3,300	3,300	3,300	3,300	3,300
Analyzed composition:						
% Crude protein	0	26.7	24.0	24.0	22.7	23.3
% Cr_2O_3	0.87	0.57	1.3	1.04	1.17	0.54

[a]Afaillin (Squibb Manufacturing Co., Manila) supplied the following in mg.kg^{-1} diet: riboflavin 53; d-calcium panthothenate 72; niacin 533; choline chloride 7,600; Vitamin B_{12} 160; procain penicillin 133; thiamin 24; pyridoxine 0.67; biotin 0.0013; inositol 0.01; para-amino benzoic acid 53; folic acid 1.2; BHT 1,200; dl-methionine 307; streptomycin 107; l-lysine HCl 273; manganese 1,293; iron 533; iodine 20; cobalt 11; copper 40; zinc 533. The following vitamins were supplied in USP, IC and IU units: Vitamin A 66,667; Vitamin D_3 26,667; and Vitamin E 27, respectively.

[b]Metabolizable energy (ME) was computed using the following energy values: 4.0 kcal.g^{-1} protein and nitrogen-free extract; 9.0 kcal.g^{-1} fat.

kcal.kg^{-1} and 3,800 kcal.kg^{-1}) in a 2 x 2 factorial design. The level of dextrin was adjusted to vary dietary energy, which was estimated using the following metabolizable energy values: protein, 4 kcal.g^{-1}; carbohydrate, 4 kcal.g^{-1}; fat, 9 kcal.g^{-1}.

Oreochromis niloticus of mixed sexes with an average weight of 13 g were held in 1.5-m^3 canvas tanks in freshwater for 15-30 days. Seventy-five per cent of the water in the canvas tanks was changed twice a week using tap water aged for 24 hours. Fish were fed to satiation twice daily with a commercial poultry feed containing 14% protein (San Miguel Corp., Manila). Fish were exposed to 2 ppm potassium permanganate for 24 hours prior to stocking in 75-l glass tanks at 20 fish each for the study.

Table 2. Composition of the protein-free diet and experimental diets prepared at 15% crude protein level (Experiment 2).

Ingredients	Diets 1	2	3	4	5
Casein	-	16.7	-	5.6	-
Copra meal	-	-	80.3	-	-
Rice bran	-	-	-	70.2	-
Azolla	-	-	-	-	77.6
Dextrin	60	45	2.3	4.4	3.4
Vegetable oil	10	10	-	-	9.0
Vitamin-mineral mix[a]	6	6	6	6	6
Cr_2O_3	1	1	1	1	1
Carboxymethyl cellulose	3	3	3	3	3
Celite	20	18.3	7.4	9.8	-
Total	100	100	100	100	100
Calculated composition (dry basis):					
% Crude protein	0	15	15	15	15
% Crude fat	10	10	10	11.7	10
% Nitrogen-free extract	60	45	44.9	41.2	38.4
% Ash	4	4	8.9	9.9	20.6
% Crude fiber	20	18.3	15.2	15.6	9.9
M.E. (kcal.kg^{-1})	3,300	3,300	3,300	3,300	3,040
Analyzed composition:					
% Crude protein	0	15.0	14.7	14.3	13.2
% Cr_2O_3	0.87	0.89	1.35	1.08	1.22

[a]Afsillin (Squibb Manufacturing Co., Manila) supplied the following in mg.kg^{-1} diet: riboflavin 53; d-calcium panthothenate 72; niacin 533; choline chloride 7,600; Vitamin B_{12} 160; procain penicillin 133; thiamin 24; pyridoxine 0.67; biotin 0.0013; inositol 0.01; para-amino benzoic acid 53; folic acid 1.2; BHT 1,200; dl-methionine 307; streptomycin 107; l-lysine HCl 273; manganese 1,293; iron 533; iodine 20; cobalt 11; copper 40; zinc 533. The following vitamins were supplied in USP, IC and IU units: Vitamin A 66,667; Vitamin D_3 26,667; and Vitamin E 27, respectively.

The glass tanks were aerated. Dissolved oxygen was 6.2 ± 1.1 ppm. Seventy-five per cent of the water was added every day to replace water removed as uneaten feeds were siphoned out. The water temperature ranged from 22°C to 26°C.

Azolla *(Azolla microphylla)* and shrimp (*Acetes* sp.) were sundried, then ground in a multipurpose grinder with 1-mm sieve. Other ingredients were sieved in a No. 20 mesh (0.013 cm) laboratory test sieve. The feed ingredients were mixed thoroughly. Water was added slowly until the mixture became dough-like and was extruded through a hand-operated meat grinder and dried in a laboratory oven at 80°C for 6 to 8 hours to approximately 5% moisture content. Prepared diets were stored in plastic jars at room temperature.

Table 3. Composition of diets prepared with two levels of fat and two levels of energy (Experiment 3).

Ingredients	Diets 1	2	3	4
Fish meal	46.6	46.6	46.6	46.6
Dextrin	21.8	8.4	43.0	33.3
Vegetable oil	1.7	7.7	3.4	7.7
Vitamin-mineral mix[a]	6	6	6	6
Cr_2O_3	1	1	1	1
Carboxymethyl cellulose	3	3	-	3
Celite	19.9	27.3	0	2.4
Total	100	100	100	100
Calculated compositon (dry basis):				
% Crude protein	30	30	30	30
% Crude fat	6	12	7.7	12
% Nitrogen-free extract	26.5	13.0	47.65	38.0
% Ash	11.4	11.4	7.4	11.4
% Crude fiber	20.1	27.6	0.23	2.6
M.E. ($kcal.kg^{-1}$)	2,800	2,800	3,800	3,800
Analyzed composition:				
% Crude protein	26.0	23.26	30.57	24.60
% Cr_2O_3	1.04	0.96	1.12	1.23

[a]Afsillin (Squibb Manufacturing Co., Manila) supplied the following in $mg.kg^{-1}$ diet: riboflavin 53; d-calcium panthothenate 72; niacin 533; choline chloride 7,600; Vitamin B_{12} 160; procain penicillin 133; thiamin 24; pyridoxine 0.67; biotin 0.0013; inositol 0.01; para-amino benzoic acid 53; folic acid 1.2; BHT 1,200; dl-methionine 307; streptomycin 107; l-lysine HCl 273; manganese 1,293; iron 533; iodine 20; cobalt 11; copper 40; zinc 533. The following vitamins were supplied in USP, IC and IU units: Vitamin A 66,667; Vitamin D_3 26,667; and Vitamin E 27, respectively.

The indirect method of using chromic oxide (Cr_2O_3) as the indigestible marker, was used to determine the digestibility of protein. The fish were fed with diets containing 0.5% or 1.0% chromic oxide for five consecutive days to ensure the presence of these diets in the gastro-intestinal tract. Fish were fed to satiation twice daily, at 8:00 a.m. and 3:00 p.m. Uneaten feeds were siphoned at 10-15 minutes after feeding. On the sixth day, the fish were sampled 6 hours after the morning feeding. They were immediately placed in ice-cold water, weighed, measured for standard length and then dissected.

Each experiment consisted of one control protein-free and/or four or five isonitrogenous, isocaloric experimental diets. The concentration of crude protein and chromic oxide were determined in the diet and feces. The apparent digestibility coefficients (ADC) for protein were computed using the formula described by Maynard and Loosli (1969):

$$\text{ADC} = 100 \cdot \frac{\dfrac{\%\ \text{Protein diet}}{\%\ \text{Marker diet}} - \dfrac{\%\ \text{Protein feces}}{\%\ \text{Marker feces}}}{\dfrac{\%\ \text{Protein diet}}{\%\ \text{Marker diet}}}$$

True digestibility coefficient (TDC) for protein was computed as follows (Kim 1974):

$$\text{TDC} = 100 \cdot \frac{\dfrac{\%\ \text{Protein diet}}{\%\ \text{Marker diet}} - \left[\dfrac{\{\%\ \text{Protein feces}}{\{\text{Marker feces}} - \dfrac{\%\ \text{Protein control}\}}{\%\ \text{Marker control}\}}\right]}{\dfrac{\%\ \text{Protein diet}}{\%\ \text{Marker diet}}}$$

As stripping was found unsuitable for collecting fecal material, intestinal dissection was used. The degree of digestion and absorption in the gut was evaluated by analyzing the protein and chromic oxide concentration of digesta collected from the stomach (section 1) and subsequent sections of the intestine in Experiments 1 and 2. The intestine was sectioned into four as shown in Fig. 1: the anterior intestine (section 2), middle intestine (section 3), and the posterior intestine which was further divided into two 5-cm sections (sections 4 and 5). Sections 4 and 5 were measured exactly; the length of sections 2 and 3 were determined by dividing the remaining portion of the intestine into two equal parts after 10 cm of the posterior portion of the intestine had been removed. Sections 2 and 3 were about 18 cm in length comprising about two-thirds of the total intestine length. In Experiment 3, only the fecal material from section 5 of the intestine was collected.

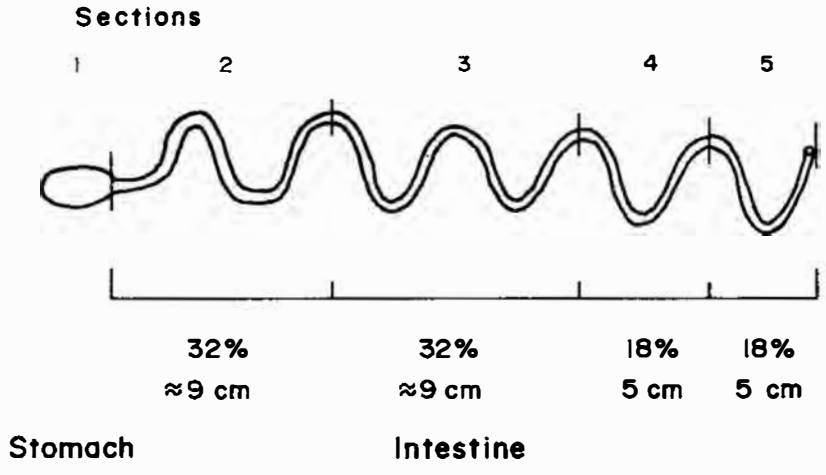

Fig. 1. Diagram of the stomach and the subsequent sections of the intestine of tilapia where fecal materials were collected. Percentage values represent the proportional length of each section in relation to total intestine length.

Each treatment was replicated three or four times within each experimental run with 20 fish/replicate in a completely randomized design. Two trials for each of the three experiments were conducted. Intestinal contents collected from fish fed with the same diet in each experimental run were pooled to collect enough fecal materials for analysis of crude protein and chromic oxide. Analyses of crude protein and chromic oxide per pooled fecal samples were done in triplicate.

Significant differences among treatments were determined using analysis of variance. Meaningful comparisons were done using Duncan's Multiple Range Test.

Proximate analyses of all feedstuffs were performed prior to diet formulation, moisture (loss in drying at 105°C for 12 hours), protein using semimicro kjeldahl flasks (kjeldahl nitrogen x 6.25), lipid (soxhlet ether extract), ash (residue after heating at 550°C for 12 hours), and nitrogen-free extract by difference (AOAC 1970). Results of the analyses are shown in Table 4.

Formulated diets and fecal materials were analyzed for crude protein using microkjeldahl and chromic oxide. Chromic oxide concentrations were determined spectrophotometrically after acid digestion in concentrated nitric and perchloric acids (Stevenson and de Langer 1960).

Table 4. Proximate analyses of feedstuffs.

Feedstuffs	Water %	Crude protein %	Ether extract %	Crude fiber %	Ash %	Nitrogen free extract %
Soybean meal	10.7	45.1	1.6	6.5	6.4	40.5
Corn gluten meal	3.7	59.5	1.2	2.6	9.7	27.0
Shrimp meal	3.0	58.7	3.2	7.6	19.8	10.7
Fish meal	6.9	64.4	9.2	0.5	15.9	10.0
Copra meal	4.8	18.7	12.5	9.7	6.1	53.0
Rice bran	7.6	14.2	16.6	8.1	8.5	52.5
Azolla	14.2	19.3	1.3	12.8	21.4	45.1

Results

Apparent digestibility coefficients (ADC) obtained from all segments of the gut of fish fed diets containing 15% protein were lower than from those fed diets containing 30% protein (Table 5). Seemingly high ADC values were observed with digesta taken from the stomach in all diets. Small increases in ADC were noticed when digesta were collected from the anterior intestine (section 2) in fish fed 30% protein while drastic decreases and negative ADC values were observed in fish fed 15% protein. Chromic oxide concentration was generally the lowest while protein content of fecal samples collected from fish fed protein-free diet was highest in this section.

Table 5. Apparent digestibility coefficients (%) for selected feedstuffs measured from subsequent sections of the gut six hours after feeding.

Protein source	Gastro-intestinal sections: Stomach 1	Anterior intestine 2	Middle intestine 3	Posterior intestine 4	Posterior intestine 5
30% Protein diets					
Casein	76.4±0.9^{c}	76.6±1.8^{c}	85.4±4.3^{b}	90.3±1.2^{a}	91.7±1.1^{a}
Corn gluten meal	64.4±2.1^{b}	77.2±0.6^{c}	84.8±2.3^{b}	89.3±0.9^{a}	90.6±1.1^{a}
Soybean meal	54.2±17.8^{ab}	64.03±3.8^{ab}	64.4±1.0^{ab}	72.9±1.4^{ab}	79.7±2.2^{a}
Fish meal	60.4±4.9^{b}	62.4±2.4^{b}	64.8±4.0^{b}	78.9±1.6^{a}	80.8±0.6^{a}
Shrimp meal	50.2±3.9^{c}	57.6±2.3^{b}	60.2±2.8^{b}	68.1±0.9^{a}	72.8±0.8^{a}
15% Protein diets					
Casein	53.5±10.9^{b}	7.0±6.8^{c}	47.6±6.4^{b}	68.7±7.3^{a}	76.7±3.0^{a}
Rice bran	36.3±6.8^{b}	-65.5±30.2^{c}	42.8±5.0^{b}	48.3±3.2^{ab}	68.9±4.1^{a}
Azolla	44.8±3.8^{a}	-26.0±15.8^{c}	22.1±5.1^{b}	32.4±6.7^{ab}	45.4±3.7^{a}
Copra meal	18.8±3.8^{a}	-209.7±31.3^{c}	-33.6±16.7^{b}	-15.0±1.6^{b}	27.0±7.3^{a}

Values represent the mean ± S.E.M. of two replicate pooled samples for sections 4 and 5; five and seven replicate pooled samples for sections 1 to 3, collected from 120 and 140 fish for high and low protein containing diets, respectively. Values bearing the same superscript in any one row are not significantly different ($P > 0.05$) from each other.

For diets containing 30% protein, small but significant ($P \leq 0.05$) increases in ADC were observed when digesta were collected sequentially from section 2 to section 3 to section 4, while no further increase was found between sections 4 and 5. On the other hand, ADC values increased greatly ($P \leq 0.01$) when digesta were taken from subsequent sections of the intestine including sections 4 and 5 for some diets with 15% protein.

The digestibility of protein was evaluated using intestinal contents collected from section 5 of the intestine (Table 6). Among the high-protein containing feedstuffs that were evaluated for *O. niloticus*, casein (97.2%) and corn gluten meal (96.7%) had the highest digestibility coefficients ($P \leq 0.05$), followed by fish meal (92.2%) and soybean meal (93.1%) which were not significantly different. Shrimp meal (86.9%) was found to be the least digestible.

Table 6. Apparent (ADC) and true (TDC) digestibility coefficients (%) of protein for high protein feedstuffs for *O. niloticus* (Experiment 1).

Protein source	Dietary protein %	Anterior Intestine (Section 2) ADC	TDC	Posterior Intestine (Section 5) ADC	TDC
Casein	26.7	76.6±1.8[a]	93.0±1.8[a]	91.7±1.1[a]	97.2±1.1[a]
Corn gluten meal	23.3	77.2±0.6[a]	92.1±0.6[a]	90.6±1.1[a]	96.7±1.1[a]
Fish meal	24.0	62.4±2.4[b]	91.9±2.4[b]	80.8±0.5[b]	92.2±0.5[b]
Soybean meal	22.7	64.0±3.8[b]	92.9±3.8[b]	79.7±2.2[b]	93.1±2.2[b]
Shrimp meal	24.0	57.6±2.3[c]	90.0±2.3[c]	72.8±0.8[c]	86.9±0.8[c]

Values represent the mean ± S.E.M. of five and two replicate pooled samples for sections 2 and 5, respectively, collected from 120 fish each. Values in the same column having the same superscript in any one column are not significantly different ($P > 0.05$).

Table 7 shows the digestibility coefficients of protein for feedstuffs with low protein content. Casein (95.6%) and rice bran (92.9%) had the highest digestibility coefficients ($P \leq 0.05$). Azolla (74.9%) was moderately digestible. Copra meal (56.4%) was the least digestible.

Apparent and true digestibility coefficients (ADC and TDC) of low protein feedstuffs significantly increased ($P \leq 0.01$) from section 2 to section 5. In some high protein feedstuffs like fish meal and soybean meal, TDC values in sections 2 and 5 did not differ significantly ($P > 0.05$).

The ADC of casein was significantly higher ($P \leq 0.05$) when incorporated in a 30% protein diet than in 15% protein diet, but TDC was not significantly different ($P > 0.05$).

Table 7. Apparent (ADC) and true (TDC) digestibility coefficients (%) of protein for low protein feedstuffs for *O. niloticus* (Experiment 2).

Protein source	Dietary protein %	Anterior Intestine (Section 2) ADC	TDC	Posterior Intestine (Section 5) ADC	TDC
Casein	15.0	7.0±6.8[a]	87.0±6.8[a]	76.7±3.0[a]	95.6±3.0[a]
Rice bran	14.7	-65.5±30.2[c]	69.5±30.2[b]	68.9±4.1[a]	92.9±4.1[a]
Azolla	14.3	-26.0±15.8[b]	59.5±15.8[c]	45.4±3.7[b]	74.9±3.7[b]
Copra meal	13.2	-209.7±31.3[d]	-75.2±31.3[d]	27.0±7.3[c]	56.4±7.3[c]

Values represent the mean ± S.E.M. of seven and two replicate pooled samples for sections 2 and 5, respectively, collected from 140 fish each. Values in the same column having the same superscript are not significantly different ($P > 0.05$).

The effect of dietary fat, carbohydrate and energy on apparent digestibility in *O. niloticus* is shown in Table 8. As dietary energy increased from 2,800 kcal.kg^{-1} to 3,800 kcal.kg^{-1}, protein digestibility decreased significantly ($P \leq 0.05$).

At the 2,800 kcal.kg^{-1} energy level, increase in fat level from 6% to 12% did not have any significant effect ($P > 0.05$) in protein digestibility. However, the 3,800 kcal.kg^{-1} diet with higher energy lipid content (12%) had a significantly ($P \leq 0.05$) lower digestibility coefficient than low fat diet (6%). The analyzed protein level in the high fat diet was the lowest resulting in a greatly reduced P:E ratio (64.7 mg.kcal^{-1}) compared with the three other diets (not less than 80 mg.kcal^{-1}).

Table 8. Effect of dietary fat, carbohydrate and energy level on apparent protein digestibility (ADC).

Diet no.	Crude protein %	Crude fat %	Dextrin %	M.E. kcal.kg^{-1}	P:E ratio mg.kcal^{-1}	ADC %
1	26.0	6	21.8	2,800	92.9	88.3±2.3[a]
2	23.3	12	8.3	2,800	83.1	89.1±1.2[a]
3	30.6	7.7	43.0	3,800	80.4	77.4±2.0[b]
4	24.6	12	33.3	3,800	64.7	68.2±2.1[c]

Values represent the mean ± S.E.M. of four replicate pooled samples obtained from 160 fish. Values with the same superscript are not significantly different ($P > 0.05$).

Discussion

It has been established that absorption of amino acids is negligible in the stomach and that substantial digestion and absorption occur in the intestine (Bowen 1982). The decrease in ADC

values for section 2 of the intestine in low protein diets may be caused by 1) the irregular movement of chromic oxide, selectively removed from the anterior intestine; 2) the significant secretions of endogenous protein such as digestive enzymes in the anterior intestine; or 3) differential gut passage for particles of different densities (Hilton et al. 1981; De Silva and Owoyemi 1983). Variations in these three factors may result not only from differences in the quantity of dietary protein and carbohydrate but also from the protein source as evidenced by significant differences ($P \leq 0.05$) among the various feedstuffs evaluated. Moreover, chromic oxide may have been selectively rejected as observed in *O. aureus* fed coarsely textured and less palatable diets such as coffee pulp and alfalfa meal diets (Popma 1982). It must be noted also that in the present experiment, the fish were not fed for 6 hours before sampling while *in vivo,* they eat more or less continuously. This atypical condition may have affected the ADC values in the stomach and the anterior intestine.

Differences in the rate of increase in ADC and TDC values along the anterior to posterior intestine were apparent not only between the 30% and 15% protein diets but also among the different feedstuffs. Among the different feedstuffs in 30% protein diet, small but significant ($P \leq 0.05$) increases in ADC were observed in the subsequent sections of the intestine without further increase between sections 4 and 5. The TDC values in the anterior intestine were comparable to those in the posterior intestine. This suggests that amino acid absorption in the anterior intestine was almost complete. On the other hand, there was a significant increase in ADC and TDC in subsequent sections of the intestine in 15% protein diets. This is most evident in copra meal which is also the least digestible protein source. Similar shift in amino acid absorption to the posterior portion of the intestine can result in incomplete digestion and absorption of gut contents and consequent underestimates of digestibility, if the contents are not collected close to the anus.

The high protein digestibility coefficients for feedstuffs both from animal and less fibrous plant materials indicated the potential for high digestive efficiency in *O. niloticus.* This was also demonstrated by De Silva and Perera (1984) in *O. niloticus.* Davis and Stickney (1978) stated that *O. aureus* fed with 36% dietary protein either from fish meal or soybean meal alone or combinations of the two feedstuffs (67% fish meal and 33% soybean meal or vice versa) gave no significant difference in growth or in body composition. The present

experiments show that digestibility of these two sources in *O. niloticus* was not significantly different.

By contrast, the digestibility of protein from soybean is significantly lower than from fish meal in carp (Dabrowski 1983) which is another herbivorous fish, and in channel catfish (Cruz 1975). Protein digestibility of fish meal (91%) in channel catfish (Cruz 1975) is comparable to that obtained for soybean meal and fishmeal in *O. niloticus* (present study).

The low digestibility coefficients (87%) for shrimp meal among high-protein containing feedstuffs may be due to high content of nonprotein nitrogen, specifically chitin. Buddington (1980) observed that *O. niloticus* has no ability to digest chitin.

The ADC of rice bran observed in this study was similar to wheat bran reported by Popma (1982), 68.9% and 70.7%, respectively. Rice bran contains low protein (10-15%) but its protein was highly available to tilapia (92% TDC). In a feeding trial on *O. niloticus*, Cruz and Laudencia (1977) observed that rations containing rice bran and fish meal gave better weight gain and best food conversion efficiency than rations with fish meal in combination with copra meal, ipil-ipil leaf meal or mulberry meal.

Based on amino acid content, azolla appears favorable as tilapia feed. Yet Almazan et al. (1986) reported lower growth performance and worsening feed conversion ratios with increased dried azolla proportions in the diet. Azolla was found moderately digestible (74.9% TDC) in the present study, but a dry, pelleted azolla-based diet was observed to be bulky, about four times the volume of similar rice bran and copra meal-based diets. The negative growth response of tilapia when fed with azolla may be due to bulkiness of the feed resulting in relatively less nutrient taken in by the fish and not due to a nutritional deficiency.

TDC values in section 5 of the intestine for casein-based diets, prepared either at 15% or 30% protein level, were not significantly different, unlike ADCs. This suggests that the former remained independent of protein level as observed by Page and Andrews (1973), Ogino and Chen (1973) and De Silva and Perera (1984). ADC is lower when dietary protein is low because the constant secretion of endogenous protein makes up a greater proportion of fecal nitrogen input which becomes progressively less as dietary protein levels increase. As a result, protein digestibility determination using ADC alone, which is commonly found in the published literature, can result in erroneous comparisons of digestibility, especially when there are significant differences in dietary protein level.

ADCs were observed to be significantly lower in diets with high dietary energy. Teshima et al. (1985) showed that at 35% dietary protein, increasing carbohydrate level from 30% to 40% improved the growth of *O. niloticus* when P:E ratio was 108 mg.kcal^{-1} but depressed growth when the P:E ratio was 85 mg.kcal^{-1}. Decrease in protein digestibility as a result of increasing dietary carbohydrate level and decreasing P:E ratio has been observed in rainbow trout by Inaba et al. (1963), Austreng et al. (1977) and Rychly and Spannhof (1979). Anderson et al. (1984) reported that growth of *O. niloticus* improved when dextrin was increased from 10% to 40% in a 35% protein diet.

High dietary carbohydrate levels adversely affect protein and energy utilization (Inaba et al. 1963; Kitamikado et al. 1964; Shimeno et al. 1978). The results in Table 8 suggest that a higher protein content in diet 3 offsets the adverse affect of high carbohydrate levels on digestibility. Further research is needed to clarify these relationships.

Increase in fat level from 6% to 12% had no significant ($P > 0.05$) effect on apparent protein digestibility at 2,800 kcal.kg^{-1} ME. Several other studies have demonstrated that dietary fat levels do not affect protein digestibility (Atherton and Aitken 1970; Kitamikado et al. 1964; Page and Andrews 1973; Dela Higuerra et al. 1977). Increasing dietary lipid can result in an increase in gross energy conversion efficiency of both dietary energy and protein (Beamish and Medland 1986; Lee and Putnam 1973) and a slight decrease in nitrogen excretion (Beamish and Thomas 1984).

The positive effect of increasing dietary lipid and carbohydrate in tilapia diets could be an effective means of reducing feed costs for tilapia production. Between the two energy sources, Teshima et al. (1985) demonstrated that based on growth, *O. niloticus* utilizes lipid more efficiently than digestible carbohydrate as an energy source when sufficient dietary protein was available. The effect of lipid and carbohydrate in the digestibility of protein in this study is consistent with this observation.

From the above, animal and plant proteins are highly available to *O. niloticus;* fats up to 12% can provide energy to spare protein without negatively affecting protein, while high carbohydrate and low P:E ratios negatively affect availability of dietary protein when dietary protein intake is limited. Thus, there is a big potential to reduce feed expenses by increasing dietary fat and carbohydrate to provide energy to *O. niloticus* and by using cheaper plant protein of comparable availability to that of animal protein.

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