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DIGESTIBILITY OF NUTRIENTS AND ENERGY IN DIETS FOR THE AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL 1822)

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Abstract

A purified diet, the raw material zein, and two diets composed of complex foodstuffs (fishmeal, soy, wheat) were tested for eight months in the African catfish *Clarias gariepinus* (Burchell 1822) to determine the *in vivo* Apparent Digestibility Coefficients (ADC) for dry matter, crude protein, lipid, carbohydrates, ash, energy, and amino acids. Feces were collected by sedimentation and digestibility coefficients were calculated using chromium oxide as an external indicator. Average ADCs ranged 54-96%. The ADC of crude protein (80-90%) was similar to previously recorded ADCs. Ash digestibility (54-89%) varied significantly between diets, probably as the result of the reduced bioavailability of minerals and trace elements in the constituent raw materials. ADC for individual amino acids ranged 82-99%. Zein, as an experimental feed ingredient, had an acceptable ADC for both gross nutrients and amino acids. When the nutrient level in the test ingredient differed greatly from that in the reference diet, calculation of ADC was based on relative nutrient contributions. Results are discussed in relation to the nature of the dietary ingredients and their suitability for related experimental nutritional work.

Introduction

The lack of data on the nutritional requirements during the various life stages of African catfish constitutes a major constraint for further development of low-cost practical feeds for this species (Wilson and Moreau, 1996). Existing knowledge of catfish nutrition cannot be considered satisfactory, as protein requirements and protein:energy (P/E) ratios using purified materials (Machiels and Henken, 1985) or complex foodstuffs (Uys, 1989) have

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been defined within quite a broad range. Further, the determination of arginine, methionine, lysine, and tryptophan requirements of juvenile African catfish (Fagbenro et al., 1998a,b, 1999; Fagbenro and Nwanna, 1999) cannot be considered conclusive because the purified diets they used were formulated with casein, gelatin, and crystalline amino acids on the basis of nutrient requirements determined by using complex foodstuffs (maize, wheat, soya, fish meal, molasses, etc). Constraints involved in this kind of food formulation include lack of precision of added nutrients and existence of uncontrolled components such as growth and anti-nutritional factors.

Diet formulation from purified materials is frequently problematic because of the imbalanced amino acid profiles of the materials used. Gelatin, which has low levels of leucine, isoleucine, lysine, methionine, and tyrosine (3.47, 1.98, 3.59, 0.39, 1.14 g/100 g crude protein, respectively), can be balanced by casein which has higher levels of these amino acids (7.18, 4.23, 6.44, 0.67, 5.16 g/100 g crude protein; NRC, 1993). Even so, the combination of casein and gelatin cannot meet the requirements of certain species for methionine and cystine (NRC, 1993). In this case, the higher levels of methionine and cystine in corn gluten (1.86 and 2.97 g/100 g crude protein, respectively) and corn zein (1.22 and 1.28 g/100 g crude protein, respectively) may enable these semi-purified materials to be used as complements to casein and gelatin in diet formulation.

Despite its high methionine and cystine content and adequate levels of other essential amino acids, the semi-purified corn by-product, zein, is low in arginine (1.98 g/100 g) and totally deficient in tryptophan and lysine (NRC, 1982). Zein has low carbohydrate and lipid levels that make it useful in experimental nutrition work where exact levels of nutrients are required (Lopez-Alvarado et al.,1994). Zein has been used as an ingredient in semi-purified diets used to determine the amino acid requirements of *Chanos chanos, Seriola quinqueradiata, Paralichthys olivaceus,* and *Pagrus major* (Borlongan and Benitez, 1990; Ruchimat et al., 1997; Forster and Ogata, 1998). However, due to its deficiency in tryptophan and lysine, zein is not easily broken down by digestive enzymes and, therefore, is often indigestible (Morrison, 1950; Clay, 1981).

The present study formed part of a more comprehensive study on refining the nutritional profile and improving the cost-effectiveness of artificial diets for African catfish, *C. gariepinus*. The tested diets were formulated for subsequent protein:energy experiments and consisted of either purified materials or complex foodstuffs. As casein and gelatin do not have optimum amino acid profiles, the digestibility of zein was investigated to evaluate its use in related nutritional experimental work.

The digestive system of fish may gradually adapt to diets, resulting in better nutrient assimilation with time. This was shown for the Asian cichlid *Etroplus suratensis* fed an aquatic macrophyte (De Silva and Perera, 1983) and milkfish *Chanos chanos* fed semi-purified diets (Ferraris et al., 1986). Although desirable in the long-term, digestive adaptation can lead to erroneous digestibility results during the transitional phase. Therefore, sufficient experimental time, replication, and allocation of different diets to different fish groups are required to counteract variability generated from digestive adaptation.

Materials and Methods

Experimental diets. Three diets were prepared. One was based on purified materials (purified diet), one on complex foodstuffs (basal reference diet), and one on a combination of complex foodstuffs and zein (zein-combined diet). The raw materials for the purified diet were carefully selected and its nutrient profile was carefully constructed to meet the diet profile needed for subsequent protein:energy experiments. The zein-combined diet and the experimental basal reference diet were produced to investigate the digestibility of zein. The composition and proximate analyses of the prepared diets are shown in Table 1.

After careful weighing, the dietary ingredients were mixed in a Hobart A200 Mixer. Oils and hot water (approximately one third of the

Table 1. Composition and nutritional profile (g/100 g dry matter) of the prepared diets.

Composition	*Purified diet*	*Basal reference diet*	*Zein-combined diet*	*Raw material zein*
Casein	31.0	-	-	-
Gelatin	6.5	-	-	-
Corn gluten	8.0	5.5	3.8	-
Corn zein	3.5	0.5	30.4	-
Fishmeal	-	26.0	18.2	-
Soya	-	15.0	10.5	-
Wheat	-	15.0	10.5	-
Dextrin	30.5	21.0	14.4	-
α-cellulose	3.0	3.0	2.1	-
Carboxymethylcellulose	0.5	1.0	0.7	-
Vegetable oil[1]	5.0	3.0	2.1	-
Fish oil[2]	5.0	3.0	2.1	-
Vitamin premix[3]	2.0	2.0	1.4	-
Mineral premix[4]	4.0	4.0	2.8	-
Chromic oxide	1.0	1.0	1.0	-
Nutritional profile				
Dry matter	94.67	95.58	95.42	95.45
Crude protein	41.66	34.25	49.55	81.38
Crude lipid	13.69	6.14	7.31	3.53
Carbohydrates	28.82	43.29	31.65	12.84
Ash	11.34	9.32	6.44	2.08
Crude fiber	4.47	7.0	4.32	0.16
Gross energy (kJ/g)	21.12	20.35	22.43	26.65
Protein:energy ratio (mg/kJ)	19.73	16.84	22.09	-
Energy:protein ratio (kJ/g)	50.67	59.4	45.27	-

[1] Pure rapeseed oil.

[2] BOCM-PAULS aquaculture grade.

[3] Composition (g/100 g premix): cyanocobalamin (B_{12}) 0.000125; ascorbic acid 3.75; cholecalciferol (D) 0.0004; tocopherolacetate (E) 0.7; vitamin K 0.15; thiamine hydrochloride (B1) 0.425; riboflavin (B2) 0.3; pyridoxine hydrochloride (B_6) 0.125; calcium pantothenate 0.525; niacinamide 1.25; biotin 0.009; folic acid 0.1; choline chloride 7.4; myoinositol 0.25; ethoxyquin 0.0019; vitamin A 0.008; α-cellulose 85.

[4] Composition (g/100 g premix): $CaHPO_4.2H_2O$ 72.77; $MgSO_4.7H_2O$ 12.75; NaCl 6; KCl 5; $FeSO_4.7H_2O$ 2.5; $ZnSO_4.7H_2O$ 0.55; $MnSO_4$.$4H_2O$ 0.25; $CuSO_4.5H_2O$ 0.078 ; $CoSO_4$.$7H_2O$ 0.047; $CaIO_3$.$6H_2O$ 0.029; $CrCl_3$.$6H_2O$ 0.012.

total weight of the prepared diet) were added. Long strings of pellets were created with a 3-mm die (food mincer attachment) and dried overnight at 40°C to a final moisture of approximately 8-10%.

The purified and semi-purified materials used for preparing the diets (casein, gelatin, dextrin, α-cellulose, carboxymethylcellulose, zein, gluten) were purchased from Sigma-Aldrich Ltd. Vegetable oil was a rapeseed pure vegetable oil while fishmeal, soybean meal (standard mechanically extracted), wheat (whole grain), and fish oil were BOCM-PAULS (Renfrew, Glasgow) aquaculture grade.

Analytical methods. Samples for water quality analyses were filtered through a Glass microfiber GF/C 2-micron filter paper and a Technicon II Autoanalyzer was used to determine total ammonia, nitrites, and nitrates using spectrophotometric methods described by Golterman (1978) for total ammonia and Strickland and Parsons (1972) for nitrites and nitrates.

Prior to analysis, samples of each diet were ground with a mortar and pestle and passed through a 1 mm sieve. All analyses were performed on a dry matter basis in triplicate. Moisture contents in raw materials and prepared diets were determined by freeze-drying to a constant weight. As feces appeared to be burnt when the standard procedure was applied (oven drying at 135°C for 2 hours; AOAC, 1990; Method 930.15), it was decided to freeze-dry all the materials (Cho et al., 1982).

The total nitrogen content in the raw materials was estimated by the Kjeldahl method and converted into crude protein as described by Pantazis and Neofitou (2003). Crude lipid, crude fiber, carbohydrates, ash, and gross energy in the raw materials and diets were also determined as in Pantazis and Neofitou (2003).

Amino acid profiles of the raw materials and diets were determined by hydrolyzing dried samples with 6N hydrochloric acid for 24 h at 110°C. After drying for 24 h over sodium hydroxide pellets, samples were dissolved in sodium citrate buffer, filtered through 0.2 μm centrifuge filters, and loaded onto an amino acid analyzer (Pharmacia LKB Biochrom Ltd., 4151 Alpha Plus model). Samples were eluted through a stainless steel (202 mm length, 0.6 mm diameter) Ultra pack 8 cation exchange resin column (sodium form) and determined spectrophotometrically by the intensity of the color produced following a ninhydrin reaction. As an external standard, a protein hydrolysate (Sigma-Aldrich Cat. No. A9781) was used containing 0.5 μmol/ml of each amino acid except for L-cystine at 0.25 μmol/ml.

Experimental design and methodology. Chromic oxide was incorporated into the diets at 1.0% as a marker to assess the digestibility of the prepared diets (Tacon and Rodrigues, 1984). The digestibility of zein in African catfish was determined by using the combined diet. This methodology was described by Wilson and Poe (1985). Having established the digestibility coefficients for the nutrients of the basal reference diet, a diet was prepared by substituting 30% of the basal reference diet with zein.

Apparent digestibility coefficients (ADC) were calculated based on the NRC (1993) formula: ADC = 100 - [100 x (%marker in feed x %nutrient in feces)/(%marker in feces x %nutrient in feed)]. This formula was also used to determine the ADC of the dry matter, after eliminating the factors “nutrient in feed” and “nutrient in feces” (Windell et al., 1978). ADC of the ingredients was calculated by equation 1 (Wilson et al., 1981): ADC = (100/30) x [digestion coefficient of combined diet - (70/100) x digestion coefficient of reference diet]. This method has advantages over using single source diets in that any synergistic effect of feeding the ingredient in combination with other diet components may be discovered and underestimating digestibility values due to decreased utilization of the single ingredient source can be prevented (Wilson et al., 1981). Equation 1 assumes that the nutrient digestibility of the combined diet is the average of the nutrient digestibility of the reference diet and the test ingredient, weighted by the proportion of each in the combined diet (70:30).

According to Forster (1999), however, equation 1 does not account for the relative contributions of the nutrient from the reference diet and the test ingredient added to the combined diet. Therefore, ADC was also computed according to formula 2 (Forster, 1999): $ADCN_{ingr} = [(a + b) \times ADCN_{com} - a \times ADCN_{ref}] \times b^{-1}$, where i = level of test ingredient in combined diet (%), a = nutrient contribution of reference diet to nutrient content of combined diet, i.e., level of nutrient in reference diet x (100 - i), b = nutrient contribution of test ingredient to nutrient content of combined diet, i.e., level of nutrient in test ingredient x i, (a + b) = level of nutrient in combined diet (%), $ADCN_{ingr}$ = apparent digestibility coefficient of a nutrient in the ingredient under investigation, $ADCN_{com}$ = apparent digestibility coefficient of a nutrient in the combined diet, and $ADCN_{ref}$ = apparent digestibility coefficient of a nutrient in the reference diet.

A limited number of fish and tanks imposed the need for sequential replication. During the eight-month experimental period, the digestibility of each diet was assessed during two of six periods. The purified diet was assessed during the first and fourth 40-day periods; the basal reference diet during the second and fifth 40-day periods and the zein-combined semi-purified diet during the third and sixth 40-day periods. Allocating two experimental periods to each diet, separated by at least one experimental period with a different diet and a one-week adaptation period, was intended to eliminate any occurrence of digestive adaptation. Feces were collected from the same individuals fed the same diet for at least two weeks to eliminate any variability in fecal chromic oxide content as a result of differential passage of this marker along the gastro-intestinal tract (Knapka et al., 1967; Bowen, 1978; Leavitt, 1985).

Culture system. The system was a specially built recirculating system consisting of twelve 10-l tanks, one 100-l header tank (2 m head) and two 100-l tanks filled with structured bio-rings (Cascade Filterpak YTH 1150 bio-rings, Mass Transfer International, Cumbria, UK) and limestone gravel to act as a biofilter (Robertson, 1992). A 3-mm mesh screen and an inverted plastic transparent funnel replaced the bottom of each 10-l tank chamber. There was a valve at the apex of the funnel for collecting feces. Water recirculated through a 75W Beresford submersible pump (Beresford Pumps, Coventry, UK) with a maximum flow rate of 3,400 l/h. Temperature was kept constant at 26-27°C by heating elements in the biofilter and header tanks.

Water was sampled fortnightly to assess water quality parameters (15 samples in total). Nitrites were 0.14±0.057 ppm, nitrates 48.5±9.85 ppm, unionized ammonia 0.66±0.11 ppm, and pH 6.6±0.047. Flow rates in individual tanks were ≤15 l/h and DO was ≥4 mg/l although oxygen is not considered a critical factor for air breathing African catfish (Haylor and Oyegunwa, 1993). The photoperiod was kept at 12:12 L:D.

Animals, husbandry and feces collection. Fish originated from the African catfish stock of the Institute of Aquaculture, University of Stirling, Scotland. Male catfish (avg wt 64.05±10.18 g) were individually stocked in the twelve 10-l chambers. Fish were fed at 19:00 by placing prepared pellets on a petri dish on the mesh screen tank bottom. After 15 min, the petri dish was removed and uneaten food was oven dried and subtracted from the given ration. Feed intakes ranged 0.5-1% of wet body weight per day. Feces were collected the following morning between 07:00 and 08:00 by opening the valve attached to the apex of the conical bottom. Feces were freeze dried and stored at -20°C until analysis.

Fecal material from each fish was pooled during each experimental period. At the end of each period, the twelve samples were randomly combined into pairs to form six samples for analysis. ADCs for each experimental period were calculated with n = 6. Final ADCs for each diet were calculated as the arithmetic mean of the pooled values for both experimental periods involved in the assessment of each diet.

Statistical analyses. The SPSS for Windows Statistical Software Package was used for statistical evaluation of results. Multiple comparisons were effected by Duncan's multiple range test (Zar, 1996).

Results

ADCs for the three experimental diets and zein are shown in Table 2. The purified diet had the highest average crude protein digestibility. There were no significant differences in crude protein digestibility between the rest of the diets or zein.

All the diets and zein had statistically insignificant ($p>0.05$) differences in crude lipid and dry matter ADCs. Carbohydrate digestibility was highest in the basal reference diet. Significantly ($p<0.05$) higher ash ADCs were recorded for the purified diet and zein.

Zein had a significantly ($p<0.05$) higher energy digestibility ADC when estimated by equation 1. Carbohydrate ADC was significantly lower and ash ADC significantly higher when estimated by equation 2. However, equation 2 did not reveal any significant differences ($p<0.05$) for the rest of nutrients between zein and the diets.

Amino ADC (Table 3) was highest for arginine, histidine, isoleucine, threonine, tyrosine, valine, aspartic acid, and glycine in the purified diet and zein. Leucine, lysine, phenylalanine, alanine, glutamic acid, and proline did not statistically differ. Except for cystine and methionine, the ADCs for the amino acids were higher in the purified diet than in the basal reference diet. The ADCs of fourteen of the seventeen (82.4%) amino acids were similar and not statistically different in zein and the purified diet. Calculation of the ADCs by equation 1 and equation 2 for zein statistically differed in only three amino acids.

Discussion

Despite the extended eight-month experimental period, the small size of the tanks used in this experiment and the applied feeding regime did not result in significantly different sized fish.

Information on the ADCs of experimental or common ingredients and their respective macronutrients for clariid catfishes has advanced in recent years. Henken et al. (1985) recorded similar ADC values for dry matter, crude protein and energy (73-83%) to those of the present experiment using adult African catfish (136.92±3.89 g) fed a diet composed of complex foodstuffs (crude protein 50%, energy 22.52 kJ/g) and using a similar feces collection system. However, their results cannot be directly compared to the results of this study due to different procedures. The Henken et al. experiment lasted only 21 days, fish were fed on a continuous nocturnal (19:00-09:00) basis at various feeding levels, and digestibility values were estimated by the direct method of quantitative collection of feces initially described by Ogino et al. (1973).

Fagbenro (1996) found various crude protein (58-92%) and energy (50-93%) ADCs for animal and plant-based foodstuffs in *Clarias isheriensis* (47.5-51.2 g) using chromic oxide as an indicator and the rectal dissection method for feces collection (Henken et al., 1985). In an effort to reduce catfish/feed production costs by replacing fishmeal, Fagbenro (1998) tested various oilseed cakes/meals in *C. gariepinus* fingerlings (47.5-51.2 g) and received crude protein (80-87%) and energy (65-79%) ADCs similar to those of the present study.

The crude protein ADCs recorded in this experiment are similar to those (70-86.6%) recorded for fingerlings (approximately 21 g) fed diets based on a mixture of algal and blood meal in various combinations with protein levels ranging 9.7-63% (Ufodike and Ekokotu, 1986). The protein digestibility of the algal and blood meal diets was directly proportional to the dietary protein level until an optimum protein ADC was reached at 50.2% dietary protein (Ufodike and Ekokotu, 1986). This was expected, since the inclusion level of algal meal in a diet affects digestibility. On the contrary, although raw materials of plant origin were included in the basal reference and zein-combined diets in this experiment at different levels, they were processed and not expected to dramatically alter the digestibility of the tested diets. As a result, the basal reference and zein-combined diets had equal ADCs for crude protein, despite their difference in crude protein levels (34% and 49.5%, respectively).

Mgbenka (1991) received lower crude protein (69.5-75%) and crude lipid (63.9-60.1%) and similar carbohydrate (65.5-73.2%) ADCs

Table 2. Apparent digestibility coefficients (%).

	Dry matter	*Crude protein*	*Crude lipid*	*Carbohydrates*	*Ash*	*Energy*
Purified diet (n = 6)						
1st experiment	78.51±2.58	92.88±1.5	96.02±0.27	67.14±3.2	89.36±1.23	82.65±0.32
2nd experiment	71.54±2.4	86.65±0.23	95.36±0.32	76.39±2.87	88.17±1.55	82.92±0.57
Average	75.02±4.92[a]	89.76±3.12[a]	95.80±0.33[a]	71.77±4.63[b]	88.96±0.59[b]	82.78±0.13[b]
Basal reference diet (n = 6)						
1st experiment	64.57±4.15	76.97±1.29	87.51±0.77	79.8±3.67	52.31±0.95	71.85±2.15
2nd experiment	71.31±3.12	88.88±1.14	95.08±0.47	85.06±2.95	55.62±0.78	78.71±0.27
Average	67.94±8.57[a]	82.93±5.9[b]	91.29±5.35[a]	82.43±2.63[a]	53.96±1.65[c]	75.28±4.85[b]
Zein-combined diet (n = 6)						
1st experiment	67.91±3.12	80.93±1.24	90.85±2.89	74.39±0.76	53.26±4.47	79.61±0.65
2nd experiment	72.79±3.84	83.06±0.55	93.71±0.90	79.25±0.93	68.12±11.84	79.85±0.56
Average	70.35±2.40[a]	81.99±1.60[b]	92.28±2.02[a]	76.83±2.4[b]	60.68±10.51[c]	79.73±0.1[b]
Calculated zein (n = 3)[1]						
1st experiment	75.70±12.73	90.25±7.78	98.65±6.88	61.75±8.57	55. 46±16.38	97.69±2.48
2nd experiment	76.25±11.27	69.43±11.95	90.52±2.07	65.72±6.90	97.30±37.66	82.51±0.63
Average	75.97±3.44[a]	79.84±14.30[b]	94.58±5.75[a]	63.73±1.98[b]	76.38±29.58[b]	90.10±10.73[a]
Calculated zein (n = 3)[2]						
1st experiment	75.57±12.63	84.78±4.05	104.16±9.93	32.49±28.41	62.93±56.95	94.83±2.20
2nd experiment	76.19±11.16	78.25±7.06	88.26±2.91	34.41±22.87	196.42±125.33	82.08±0.52
Average	75.88±3.34[a]	81.51±4.96[b]	96.21±11.24[a]	33.45±1.35[c]	129.67±94.39[a]	88.46±9.01[b]

Standard deviations are based on n = 2.
Values in the same column with different superscripts are significantly different (p<0.05).
[1] Calculation based on Wilson et al. (1981).
[2] Calculation based on Forster (1999).

Table 3. Amino acid ADC in diets and zein (n = 2).

Amino acid	*Purified diet*	*Basal reference diet*	*Zein-combined diet*	*Zein[1]*	*Zein[2]*	*SD[3]*
Essential amino acids						
Arginine	98.21±1.30[a]	91.83±0.46[b]	93.35±0.55[b]	96.85±3.41[a]	97.82±4.20[a]	3.28
Cystine (non-essential amino acid)	89.54±1.29	92.65±4.47	91.07±0.41	87.38±13.42	89.49±5.5	5.61
Histidine	97.38±1.47[a]	88.34±0.09[b]	90.83±0.73[b]	96.65±2.72[a]	95.62±2.36[a]	3.96
Isoleucine	96.10±2.16[a]	87.98±0.75[c]	90.24±0.83[c]	95.52±0.30[a]	92.41±0.60[b]	3.36
Leucine	96.02±0.51[a]	89.59±1.87[b]	90.69±0.84[b]	93.26±3.38[ab]	91.15±0.08[ab]	2.81
Lysine	97.27±1.06[a]	88.96±1.46[b]	90.38±0.06[b]	*93.71±5.03[b]	nd	4.02
Methionine	96.68±1.12[b]	96.97±0.3[ab]	97.52±0.53[ab]	98.79±0.74[a]	98.52±0.69[a]	1.03
Phenylalanine	95.78±3.04[a]	82.96±1.96[b]	85.91±0.61[ab]	92.79±8.50[ab]	87.72±2.69[ab]	5.90
Threonine	97.05±2.05[a]	89.22±1.07[c]	91.17±0.96[bc]	95.71±0.29[a]	93.87±0.22[ab]	3.16
Tyrosine (non-essential amino acid)	97.33±1.60[a]	87.91±0.23[c]	90.63±0.50[bc]	96.98±2.40[a]	92.37±1.01[b]	3.98
Valine	97.55±0.43[a]	87.27±0.57[c]	89.78±0.49[bc]	95.62±3.54[a]	93.55±2.46[ab]	4.23
Non-essential amino acids						
Alanine	96.47±1.66[a]	86.78±1.39[c]	89.26±0.55[bc]	94.36±3.67[ab]	90.13±0.52[bc]	4.03
Aspartic acid	96.48±1.36[a]	87.84±0.01[c]	91.06±0.25[b]	98.58±0.89[a]	96.17±0.69[a]	4.22
Glutamic acid	98.64±0.46	91.98±0.82	92.10±0.90	92.36±5.71	92.18±2.34	3.46
Glycine	97.87±1.05[a]	86.99±0.10[c]	90.16±0.37[b]	97.55±0.89[a]	99.46±1.03[a]	5.20
Proline	97.20±0.79[a]	92.10±0.43[b]	89.13±0.85[c]	82.21±1.43[d]	87.3±1.0[c]	5.29
Serine	96.89±1.70[a]	87.23±1.54[c]	89.19±0.60[bc]	93.87±2.84[ab]	91.11±0.85[bc]	3.85

Values in the same row with different superscripts are significantly different (p<0.05).

[1] Calculation based on Wilson et al. (1981).

[2] Calculation based on Forster (1999).

[3] Standard deviation of the multiple comparisons.

* Zein is totally deficient in lysine of endogenous origin. The lysine ADC value is due to lysine contained in the feces that did not originate from zein but from endogenous excretions of the digestive tract.

nd = not determined since coefficient b in Forster's formula 2 equals zero.

for agricultural by-products (plantain peels - *Musa sp.*, yam peels - *Dioscorea sp*.) and fish waste fed to juvenile African catfish. The ADCs for crude protein in the diets and zein in our experiments were higher than those reported for moist diets based on fermented tilapia silage and tested in *C. gariepinus* of an average weight of 18.5±1.3 g (Fagbenro and Jauncey, 1994). Dry diets based on co-dried fermented tilapia silage and soybean flour blended with fishmeal and corn starch and tested in *C. gariepinus* (10.8±0.3 g; Fagbenro, 1994; Fagbenro et al., 1994) had almost equal crude protein and energy ADCs to those reported in this study.

Protein availability in diets containing soy may be affected by the presence of soy crystalline globular proteins that act as trypsin inhibitors (Liener and Kakade, 1980). Both the basal reference and the zein-combined diets contained soy at levels that might have had a negative effect, explaining the higher ADCs for crude protein and most of the amino acids in the purified diet. However, levels of trypsin inhibitors in the commercial aquaculture grade soy used in these experiments should have been low (Jauncey 1999, pers. comm.).

Although the Cr_2O_3 level in the diets was above 2%, it is possible that a faster (relative to digesta) Cr_2O_3 transit rate took place, resulting in overestimation of crude lipid ADCs (Ringo, 1993a,b). However, the experimental design should have eliminated this component of variation.

The low ash ADC values for the basal reference and zein-combined diets could have been the effect of the low bio-availability of minerals and trace elements in their constituent raw materials. The apparent availability of phosphorus in whole-fish meals for salmonids ranges 20-70% according to the form of phosphate salt (monobasic, dibasic, tribasic) and ash (bone) content in the fishmeal (Yone and Toshima, 1979; Lall, 1991; Riche and Brown, 1996; Nordrum et al., 1997; Yamamoto et al., 1997). In plant proteins, a large proportion of phosphorus is present as organically bound phytate. Phytic acid phosphorus is largely unavailable, has the capacity to chelate other trace elements (i.e., iron, copper, zinc, cobalt, molybdenum), and, by so doing, may render them biologically unavailable to fish during digestion (Spinelli, 1980; Lovell, 1989; Hossain and Jauncey, 1991). Both the basal reference and zein-combined diets contained fishmeal and grain meals (soy, wheat) whose minerals might have been biologically unavailable, resulting in the lower ash ADC compared to that of the purified diet.

The significant difference in zein ADCs for carbohydrates and ash using equation 2 compared to equation 1 was mainly caused by the disparity in values of those two nutrients between the basal reference diet and zein. This disparity resulted in very unequal nutrient contributions (a, b, in equation 2) of each component (reference diet-zein) to the nutrient level (a + b, in equation 2) of the zein-combined diet (Forster 1999). Another source of variation between the ADC values obtained by the two equations for the zein nutrients is the difference between the nutrient ADC values of the basal reference diet and the zein-combined diet.

The energy ADC for zein based on equation 1 is slightly but significantly higher ($p<0.05$) than that based on equation 2. This does not completely reflect the digestibility value of the energy component, as the ADC of protein and lipid were similar according to the two equations but carbohydrate ADC using equation 1 almost doubled that calculated using equation 2. The reasons for this are not clear. The lack of significant differences ($p<0.05$) between zein ADC for dry matter and crude lipid, calculated according to the two equations, can be attributed to the minimal difference in a and b values for those nutrients. Further, the lack of statistical difference in ADC for all except three amino acids as estimated by the two equations can be attributed to minor differences in amino acid values between zein and the basal reference diet.

This study showed that calculation of nutrient ADC in a single ingredient by Forster's method significantly differs from those calculated using the traditional Wilson method, particularly when nutrients of the test ingredient are significantly lower than in the basal reference diet.

Dextrin, an intermediate product of starch hydrolysis to maltose and *d*-glucose, is characterized by similar glycoside linkages to starch and was the prevalent carbohydrate source in the purified diet. The relatively high carbohydrate digestibility for this diet (71.77±4.63%) suggests that starch digestibility in African catfish is high and confirms the hypothesis of Uys (1989) that the good starch utilization in this species is due to elevated amylase levels in the anterior part of the intestine.

The higher ADCs for crude protein and most amino acids in the purified diet, coupled with equally high ADCs for other nutrients and good palatability, indicate that the purified experimental diet can be an efficient tool for assessing assimilation efficiency in African catfish. As digestibility *per se* does not reveal anything related to the time-course of absorption, further investigation of the serum free amino acid concentrations following the administration of such diets will better elucidate their intra-specific amino acid relationships and their effect on protein utilization.

Maize, which has a high zein content, is usually characterized by low protein digestibility since zein is not easily broken down by enzymes due to its absence of lysine and tryptophan (Morrison, 1950; Clay, 1981). However, the zein used in this experiment was semi-purified and had no apparent negative effect on the digestive process. The ADCs of nutrients and amino acids in both the zein-combined diet and zein as an ingredient were quite acceptable ADCs. As 82.4% of zein amino acids had similar and not statistically different ADCs to those of the purified diet, use of zein in related nutritional experimental work can be recommended. Further, the zein ADC can be used as a broadly applied index of zein digestibility for African catfish.

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