CRITIQUE AND REVIEW OF METHODS OF DIGESTIBILITY EVALUATION IN NUTRITION STUDIES -DIGESTIBILITY OF SOYBEAN FLOUR, POULTRY MEAT MEAL AND WHEAT FLOUR IN *OREOCHROMIS NILOTICUS* AND *CLARIAS GARIEPINUS* AS CASE STUDIES.

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ABSTRACT

The digestibility of soybean flour (SF), poultry meat meal (PMM) and wheat flour (WF) in *Oreochromis niloticus* and *Clarias gariepinus* were investigated. There was no significant difference in the nutrient apparent digestibility coefficient (ADC) of the test and reference diets (p>0. 05). The dry matter, protein, lipid and ash ADCs were high in the diets and that all test diets were as digestible as the reference diet. The protein of SF test diet was more digestible than that of the reference diet in *O. niloticus.* Similarly, the dry matter and the ash of PMM test diet were more digestible than those of reference diet in *0. gariepinus.* These were about 100% digestible. 30% replacement of the reference diet gave a test diet whose proximate composition differed markedly with that of the test feedstuff being evaluated and insignificantly with that of the reference diet. This was considered inappropriate and therefore suggested that a much higher level of replacement be considered in digestibility study.

Keywords: Digestibility, Soybean flour, Poultry meat meal, wheat flour, Oreochromis niloticus, Clarias gariepinus.

INTRODUCTION

A basic step to choice of feedstuffs is knowing the digestibility of the feedstuffs. This has been necessitated by the quest for the development of low-cost diets using agricultural by-products in fish culture (De Silva, et al., 1990). Most of these are non-conventional and will therefore demand characterization by biological and chemical evaluation. It is also an indicator of potentially available energy and nutrients for

maintenance, growth and reproduction of the animal and a measure of indigestible nutrients that account for major portion of waste generated from aquaculture operation (Cho, C.Y., 1991). The digestibility coefficients of feedstuffs in a diet are unaffected by biotic and abiotic factors, and additive. (Cho and Kaushik, 1990). This has made possible the development of an arithematical and predictive model for the digestibility of innumerable diet formulations possible from such feedstuffs.

Several methods have been developed for feedstuffs digestibility investigation. Cho et al.,. (1985) replaced 30% of reference diet with the test ingredient in the test diets. De Silva et al., (1990) found that mixing 15-20% of the ingredient (leaf meal) to a reference diet would be more desirable in determining the digestibility of leaf meal in *Oreochromis aureus*. However, their results showed very high apparent digestibility coefficients (ADC) for test diets with 10 and 20% inclusion levels of the test ingredient. There were cases at 10% where inclusion level gave higher ADC of test diet than the reference diet signifying >100% digestibility which is theoretically impossible. Value obtained at 20% and above guaranteed <100% ADC values.

This controversy still requires further investigation into the suitability of the conventionally used Cho et al. (1985) formulation. As a result of the foregoing, 30% inclusion level of ingredients, viz; soybean flour, poultry meat meal and wheat flour into a reference diet containing fish meal and wheat flour to give the test diet was adopted for this investigation.

MATERIALS AND METHODS

Experimental Analyses

Four reference diets (4.2 $kcalg^{-1}$ energy and 38% crude protein) containing fish meal as protein supplement and wheat flour were formulated. soybean flour, poultry meat and meal and wheat flour were used to individually replace 30% of each of three of the reference diets to give three test diets. These were fed to *Oreochromis niolticus* (M±S.E. = 21.6±0.6g and *Clarias gariepinus* (M±S.E. = 50.0±1.8g at 2% body weight per day at 11.00 hr for four weeks.

Table 1: Ingredients and proximate composition of reference and test diets used in feed stuff digestibility study

Diets	Ref: Diet (RF)	Test Diet (RF+SF	Test Diet RF+PMM	Test Diet (RF+WF)
Fish meal	52.4	36.7	36.7	36.7
Soybean flour	-	30	-	-
Poultry meat meal	-	-	30	-
Wheat flour	31.9	22.3	22.3	52.3
Soybean oil	7.8	5.5	5.5	5.5
Vitamin premix[1]	2.0	1.4	1.4	1.4
Mineral premix[2]	4.0	2.8	2.8	2.8
Binder (CMC)[3]	1.5	1.1	1.1	1.1
Cr_2O_3	0.5	0.4	0.4	0.4
Proximate composition				
Moisture	8.5	7.6	6.2	6.3
Protein	38.2	40.2	44.4	31.7
Lipid	13.4	10.9	13.0	11.0
Ash	9.5	8.5	10.9	7.7
Energy ($kaclg^{-1}$)	4.6	4.6	4.7	4.6

[1]vitamin premix providing the following vitamins ($mgKg^{-1}$ premix): vitamin A, 1000; vitamin D, 4.0; vitamin E, 7000; vitamin K, 1500; vitamin C, 37500; thiamine, 4250, riboflavine, 3000; pyridoxine, 1250; pantothenic acid, 5250; Niacin, 12500, biotin, 90; folic acid, 1000; vitamin $B_{12,}$ 1.25; choline, 74050; inositol, 25000;

[2]Mineral premix providing the following minerals (gkg^{-1} premix): calcium orthophosphate, 727.8; magnesium sulphate, 127.5; sodium chloride, 60; potassium chloride, 50; iron sulphate, 25; zinc sulphate, 5.5; manganese sulphate, 2.5; copper sulphate, 0.8; cobalt sulphate, 0.5; calcium iodate, 0;3; chromic chloride, 0.1.

[3]CM (Carboxymethyl cellulose).

Table 2: Total dry matter (TDM) and protein digestibility coefficients of leaf meal fed to *Oreochromis aureus* (after De Silva *et al.*, 1990).

	Total	Dry	Matter	Dig.	Prot.	Dig.		
	Cr_2O_3	marker	CF*	marker	Cr_2O_3	marker	CF*	marker
	Daytime	Night	Daytime	Night	Daytime	Night	Daytime	Night
RD	73.4±2.4	78.9±2.4	80.0±1.8	78.9±2.8	83.8±3.6	86.8±1.9	87.9±1.9	87.1±2.7
RD+10% LM	74.2±2.3 (100.1)	77.9±2.2 (99.9)	78.2±2.0 (99.7)	76.1±1.6 (99.6)	84.1±2.6 (100.0)	87.1±1.4 (100.0)	86.6±1.1 (99.8)	86.5±1.0 (99.9)
RD+20% LM	68.2±0.3 (99.2)	71.5±5.0 (98.9)	73.3±1.7 (99.0)	71.7±1.3 (98.9)	81.4±0.5 (99.7)	83.5±2.3 (99.6)	83.8±0.5 (99.5)	83.6±0.9 (99.5)
RD+30% LM	63.4±4.6 (98.3)	67.2±5.6 (98.1)	68.3±0.9 (98.1)	69.1±1.1 (98.4)	76.4±2.7 (98.9)	80.4±3.1 (99.2)	79.8±0.5 (98.9)	81.8±0.5 (99.3)
RD+40% LM	60.8±1.7 (97.7)	65.5±8.8 (97.7)	63.4±0.6 (97.1)	63.7±1.2 (97.4)	73.9±2.2 (98.5)	77.9±4.5 (98.7)	75.7±1.4 (98.2)	77.0±1.0 (98.5)
RD+50% LM	55.7±2.3 (96.5)	59.4±7.3 (96.4)	55.8 (95.2)	59.3±1.2 (96.3)	68.8±2.4 (97.6)	71.7±4.4 (97.7)	68.9±1.5 (96.9)	71.7±1.4 (96.9)

*CF, Crude fibre

Data in parenthesis represents digestibility coefficients of leaf meal.

Uneaten food was siphoned out at 1:00Hr and faecal collection made at 10:00 hr before next feeding. Fishes were stocked at 10 per 50L tank in a recirculatory system with water flow rate of 0.5L per minute with recirculation effective from the top. Water quality parameters were monitored thus: Temperature, 26-27°C dissolved oxygen (DO), 4.4-6.2 mgl^{-1}; pH, 6.0 - 6.5; NH_3. N, 0.2-0.6 mgL^{-1}; $N0_2$.N, 0.2 mgL^{-1}; NO_3.N, 10-20 mgL^{-1}; Ca-hardness, 51-62 mgL^{-1} and total hardness, 59-71 mgL^{-1}.

Proximate analysis of feedstuffs, diets and faecal samples was performed according to AOAC (1990). Moisture was by oven-drying, protein by micro-kjeldahl technique using kjeldahl Auto 1030 Analyzer, lipid by solvent extraction with soxtec system 1043 Extractor and ash by incinerating in a Muffle furnace. Chromic oxide analysis was according to Furukawa and Tsukahara (1966). Apparent digestibility coefficient of diets was evaluated according to Bondi (1987) and that of feedstuffs was calculated according to Cho, *et al.*, (1985) as given below:

$$\text{ADC9\%) of nutrients} = 100 - \left(100 \frac{Cr_2O_3 \text{ in diets}}{Cr_2O_3 \text{ in faces}} \times \frac{\text{nutrients in faeces}}{\text{nutrient in diet}}\right)$$

$$\text{ADC Test ingredient} = \frac{\text{ADC of test diet} - 0.7 \text{ ADC of ref. diet}}{0.3 \text{ ADC of test diet.}}$$

Statistical Analyses

Data were subjected to one way Analysis of Variance (ANOVA), multifactor ANOVA and Multiple Range Test (Steele and Torrie, 1960) for variance analysis, means comparison and interactions of factors. Percentage data were transformed by arc-sine transformation (Zar, 1984) prior to analysis.

RESULTS

i. **Experiment 1** *Oreochromis niloticus*

The dry matter, protein, lipid and ash apparent digestibility coefficients (ADC) of the reference diet and test diet are presented in Table 3. The dry matter, protein, lipid and ADC were high in test diets containing SF, PMM and WF in decreasing order except that of PMM that was highest for ash. These were as almost as digestible as the reference diet. Infact the protein of soybean was more digestible. The nutrient ADC of SF, PMM and WF in parenthesis confirm this observation as they were above 80% for all the nutrients evaluated and that of soybean protein was >100%.

Table 3: Total dry matter, protein, lipid and ash digestibility coefficients of SF, PMM and WF fed to *Oreochromis niloticus*

Diets	Total dry matter digestibility	Protein digestibility	Lipid digestibility	Ash digestibility
Reference diet (RD)	96.7±0.3	85.0±1.2	97.0±1.4	92.3±0.1
RD+30% Soybean	96.3±0.3 (98.9±0.8)	87.4±0.0 (106.3±0.1)	96.3±0.9 (98.3±2.2)	86.9±1.1 (85.5±3.2)
RD+30% Poultry meat meal	95.3±0.0 (96.6±0.1)	84.9±0.9 (99.7±2.3)	95.6±0.3 (96.6±0.7)	88.4±0.0 (89.8±0.0
RD+30% Wheat flour	93.7±0.00 (92.5±0.0	80.7±1.0 (87.5±3.1)	93.1±2.2 (90.2±5.7	86.8±1.3 (85.3±3.6

Data in parenthesis represent the digestibility coefficients of SF, PMM and WF.

ii. **Experiment 2** *Clarias gariepinus*

Table 4 depicts the dry matter, protein, lipid and ash apparent digestibility coefficients (ADC) of the reference diet, test diets and the feedstuffs. The dry matter, protein, lipid and ADC were high in test diets containing SF, PMM and WF. Dry matter, protein and ash ADC were highest in PMM test diet while lipid was highest in SF test diet. These were almost as digestible as the reference diet. Infact the dry matter and ash were more digestible. The nutrient ADC of SF, PMM and WF in parenthesis are in agreement with this observation as they were above 80% for all the nutrients evaluated and that of PMM dry matter and ash were >100%

Table 4: Total dry matter, protein, lipid and ash digestibility coefficients of SF, PMM and WF fed to *Clarias gariepinus*

Diets	Total dry matter digestibility	Protein digestibility	Lipid digestibility	Ash digestibility
Reference diet (RD)	93.3±0.0	90.4±0.0	91.1±0.0	98.4±0.0
RD+30% Soybean flour	91.2±0.5 (94.8±1.4)	84.2±0.0 (82.8±0.1)	93.1±0.2 (105.1±0.5)	98.2±0.4 (99.6±0.9)
RD+30% Poultry meat meal	95.8±0.0 (106.1±0.1)	85.4±0.1 (87.3±0.3)	88.3±2.3 (88.6±0.1	98.8±0.0 (101.1±0.0)
RD+30% Wheat flour	92.3±0.2 (97.6±0.6)	81.9±0.6 (75.9±1.8)	87.0±0.1 (92.6±6.4	98.3±0.1 (99.9±0.1

Data in parenthesis represent the digestibility coefficients of SF,

DISCUSSION

Very high nutrient digestibility were observed in this investigation. The choice of fish meal as the sole protein supplement in the reference diet gave high dry matter, protein, lipid and ash apparent digestibility coefficients. However, test diets containing 305 replacement of reference diet with the feedstuffs also gave high nutrient ADC. Consequently, the feedstuff apparent digestibility coefficients which are derivatives of reference and test diet ADCs were equally high.

The >100% ADC recorded for SF protein in *O. niloticus* was not unconnected with fact that it has been reported to be equally or more digestible than fish meal in some fishes. The protein of dehulled, solvent-extracted soybean meal containing 49% crude

protein was reported to be 85% digestible to channel catfish (Lovell, 1977), rainbow trout (Smith, 1976) and tilapia (Poma, 1982) and that these ADC were equal to or higher than those for whole fish meal protein (Lovell, 1990).

The excessively high dry matter and ash ADC for PMM in *C. gariepinus* was as a result of higher dry matter and ash ADC for test diet containing PMM than reference diet. De Silva *et al,* (1990) reported high dry matter and protein ADC in *Oreochromis aureus* fed leaf with even higher ADC for dry matter in night faecal collection and protein in daytime faecal collection at 10% level of replacing reference diet with test feedstuff.

The excessively high feedstuffs digestibility observed in this investigation was attributed to reference diet type and level of its replacement by the feedstuffs in the test diets. The conventional use of fish meal which lacks absolute digestibility superiority over other high quality feedstuffs like dehulled, toasted and solvent extracted soybean meal needs to be reconsidered.

30% replacement level is from all indication inadequate as all test diets were almost as digestible as the reference diet even that containing wheat flour. This level was probably insignificant to effect any change. A significantly high level of inclusion say 70% that would make the test diet share insignificantly different nutritive value with the feedstuffs rather than reference diet would be most appropriate. The feedstuff ADC results from this investigation were more representative of fish meal ADC in the reference diet than those of SF, PMM and WF in the test diets.

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