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Apparent Digestibility Coefficients of Differentially Processed Mucuna Cochinchinensis (Lour.) Seed Meal by Hybrid Catfish (*Heterobranchus Longifilis X Clarias Gariepinus*) Fingerlings

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Abstract

A trial was conducted using static water in circular plastic aquaria to determine the apparent digestibility coefficients (ADCs) of crude protein and gross energy of processed mucuna seed meal (MSM) by hybrid catfish. A reference diet comprising casein (32%), gelatin (8%), starch (40%), fish oil (10%), vitamin/mineral premix (9%) and chromic oxide (1%) and four other diets formulated with each type of processed MSM constituting 30% of the reference diet were used. All diets were isonitrogenous and isocaloric. Each diet was assigned to three aquaria each of which contained ten fish (mean weight 5.5 ± 0.5g). Feeding was twice daily at 5% body weight. Low ADC crude protein values were obtained for MSM diets when compared with similar legume seed meals. However, boiled dehulled MSM gave the highest ADC crude protein that was significantly different from other MSM diets. Similarly, boiled dehulled MSM had the highest ADC gross energy that was significantly different from other MSM diets and compared with the values for similar legumes. It suggested that higher ADC crude protein may be achieved by supplementing boiled dehulled MSM with deficient essential amino acids.

Keywords: Hybrid catfish (*Heterobranchus longifilis x Clarias gariepinus*), Diets, Nutrient digestibility, *Mucuna cochinchinensis*.

1.0 Introduction

Fishmeal is the main dietary protein source for most aquaculture species. It constitutes about 40% of aquaculture production (Fagbenro 1998). However, in the near future, the production of fishmeal will not be enough to cover the increasing demand (Tacon 1997; Siddhuraju and Becker,2001). This has prompted research aimed at replacing the fishmeal with locally available and cheap plant materials (Fagbenro 1998; Siddhuraju and Becker 2001; Osuigwe *et al*. 2002). According to Rumsey (1993), increased use of plant protein supplements in fish feeds will reduce the cost of fish feed. Oil seed meals such as soybean products have been used to replace a large proportion of proteins in fishmeal (Lovel 1988; Lim and Akiyama 1992).Utilization of a wide range of conventional legumes as protein sources for fish feed are limited because of its increasing demand for use for human consumption and as input in animal feed industries (Siddhuraju and Becker 2001). This underlines the need that less expensive and readily available plant protein sources should replace fishmeal or other exotic feed ingredients without diminishing the nutritional quality of fish feed (National Research Council (NRC) 1983; El Sayed 1999).

M. cochinchinensis is a legume that is widely available and has similar nutritional qualities as soybean and other conventional legumes because it contains similar proportions of protein, lipid, minerals and other nutrients (Ukachukwu and Obioha 1996; McCallum 2004) and its potential as a dietary protein and energy source for animal feed is well recognized especially in developing countries (Duke 1981).

One of the most important characteristics of feed stuffs is their digestible crude protein and digestible energy value. Information on the digestible coefficients of macro nutrients in common ingredients for hybrid catfish (*H.longifilis x C.gariepinus)* is limited. The objective of this study was to determine the Apparent Digestibility Coefficient (ADC) crude protein and gross energy for *M.cochinchinensis* seed meal by fingerlings of hybrid catfish, a generally preferred culture catfish.

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2.0 Materials and Methods

M.cochinchinensis seeds were milled to fine powder using a locally fabricated attrition mill. One half of the milled MSM (Mucuna Seed Meal) was sieved using a fine (250µm) net to remove the seed coat of the milled Mucuna. The sieved portion served as dehulled MSM while the portion that was not subjected to sieving was regarded as the undehulled. One half of each portion of the dehulled and undehulled seed meals were moistened, wrapped with thermostable polythene sheets and boiled for 60 minutes in water. Thereafter, they were oven dried at 60^{0}C for 10 hours. The other halves were left raw. Thus, four types of MSM were obtained as follows:

(i) raw undehulled
(ii) raw dehulled
(iii) boiled undehulled
(iv) boiled dehulled.

These were appropriately labeled for subsequent formulation of the test diets. Sub samples were labeled and stored at -20^{0}C and later used for proximate composition analyses for dry matter, crude protein, ether extract, crude fibre and total ash using methods described by Official Methods of Analysis of the Association of Official Analytical Chemists (A.O.A.C. 1990). Gross energy was calculated using 4.1, 5.4 and 9.5 to multiply carbohydrate (NFE), protein and lipid respectively (Jobling, 1983). Essential amino acid composition was determined by the method of Adebowale *et al*. (2005).

A reference diet was formulated using casein, gelatin, cassava starch, fish oil, mineral/vitamin premix and chromic oxide at 32%, 8%, 40%, 10%, 9% and 1% inclusion levels respectively (Fagbenro 1998). The test diets containing 70% of the reference diet and 30% of the differently processed MSM were prepared (Table 1). The diets were prepared by thoroughly mixing the dry ingredients in a mixer before oil and finally water (400ml kg^{-1} diet) was added. The moist mixture was passed through a grinder with a 5mm diameter die to produce pellets which were thereafter oven dried at 70 ^{0}C to a moisture content of 10% (Fagbenro 1999). The dry pellets were crumbled to obtain 1cm long pellets which were then stored at -20^{0}C in air-tight polyethene bags until used for feeding.

Hybrid catfish *(Heterobranchus longifilis x Clarias gariepinus*) fingerlings (mean weight 5.5 ± 0.5 g) were acclimated for seven days and randomly distributed in groups of ten fingerlings into 25 litre circular aquaria containing rain water that was completely replaced every three day. The average water temperature, pH and dissolved oxygen (DO) during the culture period were 28 ^{0}C, 6.8 and 5.7 mg/L respectively. Each diet was randomly assigned to triplicate aquaria and the fish were fed at 5% body weight twice daily (08.30 – 09.00 hr and 17.00 – 17.30 hr) for fourteen days before the commencement of the collection of faeces.

About four hours after administering feed, all feed particles were removed from the aquaria by siphoning. Before the next administration of feed, faecal materials were similarly collected from each aquarium, dried and stored (-20^{0}C) in labeled containers until enough quantity was collected for proximate composition analysis. Triplicate samples of feed and faeces were then analysed by A.O.A.C. (1990) methods for proximate analysis. Gross energy was determined by computation (Jobling 1983) while chromic oxide content was determined using the methods of Furukawa and Tsukahara (1996). Apparent Digestibility Coefficients (ADC) for crude protein and gross energy in the test diets were calculated as follows:

$$\text{ADC Protein} = 10^2 - [10^2 \times (l_d / l_f \times N_f / N_d)] \quad ...(1)$$

$$\text{ADC gross energy} = E_d - [E_f \times I_d / I_f] \quad ...(2)$$

Where l_d represents chromic oxide in diet and l_f represents chromic oxide in faeces, N_d is protein in diet, N_f is protein in faeces, E_d denotes gross energy of diet and E_f is gross energy of faeces.

The ADC protein and ADC gross energy in the test feed stuff were calculated based upon the 70:30 ratio of the reference diet mixture and test feedstuff in each of the test diets as follows:

$$\text{ADC nutrient} = 100/30\,[\text{ADC test diet} - (70/100) \times (\text{ADC ref diet})] \quad ...(3)$$

Data from the analysis were subjected to Analysis of Variance (ANOVA). Differentiation of means was carried out using Duncan's Multiple Range Test with SPSS for Windows (1999).

Table 1: Formulation (g 100g^{-1}) of Reference and Test Diets.

Ingredient	Reference Diet	Test Diet
Casein	32.00	22.40
Gelatin	8.00	5.60
Starch	40.00	28.00
Mineral/Vitamin premix	9.00	7.00
Fish oil	10.00	6.30
Chromic oxide	1.00	0.70
*Feedstuff	-	30.00

* Feedstuff were either undehulled boiled, undehulled raw, dehulled raw or dehulled boiled. *Mucuna cochinchinensis* seed meal.

Table 2: Proximate composition (g 100g^{-1}), gross energy (Kcal kg^{-1}) and essential amino acids (g 100g^{-1}) of differently processed *M.cochinchinensis* seed meal (MSM).

	Undehulled raw MSM	Dehulled raw MSM	Undehulled boiled MSM	Dehulled Boiled MSM
Proximate composition				
Dry matter	93.40^{a}	92.46^{b}	91.83^{c}	91.25^{d}
Crude protein	31.00^{c}	33.43^{a}	30.64^{d}	32.22^{b}
Ether extract	7.35^{b}	6.22^{d}	8.10^{a}	6.91^{c}
Crude fibre	5.23^{a}	4.36^{b}	4.22^{b}	4.01^{b}
Total ash	3.28^{ab}	3.63^{a}	3.19^{b}	3.11^{b}
NFE	51.14^{c}	52.36^{b}	53.85^{a}	53.75^{a}
Gross energy	4468.99^{a}	4542.88^{a}	4631.91^{a}	4600.08^{a}
*Essential amino acids				
Arginine	3.09			
Histidine	0.91			
Isoleucine	3.48			
Leucine	2.79			
Lysine	2.60			
Methionine	0.51			
Phenylalanine	2.95			
Threonine	1.94			
Tryptophan	0.89			
Valine	2.68			

Means on the same row with different subscripts are significantly different ($P<0.05$). NFE= Nitrogen Free Extracts. * Adapted from Adebowale et al., (2005)

3.0 Results

The proximate composition of the differently processed MSM is shown in Table 2. Dehulling increased the crude protein of MSM significantly over the undehulled meals. On the other hand, boiling significantly decreased the crude protein values of the meals when compared to the raw meals. Both dehulling and boiling significantly reduced the crude fibre levels of the seed meals. Boiling significantly increased the nitrogen free extracts (NFE) values of the MSM when compared to the raw MSM. However the processing methods had no significant effect on the gross energy (GE) values of the seed meals which remained similar. The raw MSM is deficient in methionine an essential amino acid.

Table 3 shows the mean apparent digestibility coefficients (ADCs) of protein and gross energy for the test diets. ADC protein for the control diet was highest (93.33 %) and significantly different from other diets. Amongst the diets containing MSM, the ADC protein for diet containing dehulled boiled MSM had the highest value (56.19 %) that was significantly different from others which were similar.

Table 3: Mean apparent digestibility coefficients (%) for crude protein and gross energy in differently processed *M.cochinchinensis* seed meal by hybrid catfish.

Ingredient	ADC(%)			
Seed meal Type	Protein	Energy	DP*	DE**
Control	93.33^{a}	69.20^{ab}		
Undehulled raw	40.17^{c}	41.16^{c}	12.45	1839.44
Undehulled boiled	46.28^{c}	63.09^{b}	14.18	2922.27
Dehulled raw	46.25^{c}	38.30^{c}	15.46	1739.92
Dehulled boiled	56.19^{b}	79.50^{a}	18.10	3657.00

Means with the same superscripts on the same column are significantly different ($P<0.05$); *Digestible Protein (g 100^{-1}) ** Digestible Energy (Kcal Kg^{-1}).

On the other hand the ADC gross energy for dehulled boiled MSM had the highest value (79.50 %) which however was similar to the values of the control which was as well similar to undehulled boiled MSM. The raw MSM diets had low ADC gross energy values that were similar.

4.0 Discussion

Mucuna seeds have thick leathery skin (Ene-Obong and Carnovale 1992). The increase in the crude protein content of the seed meal as a result of dehulling (Table 2) was therefore expected bearing in mind that generally the seed coat contains less crude protein than the cotyledon (Adebowale et al. 2005). Dehulling thus not only increased the crude protein content of the MSM significantly but also reduced the crude fibre content.

The reduction of the crude fibre content of the boiled MSM in this study agreed with the report of Ukachukwu and Obioha (1997) for *Mucuna cochinchinensis*. This may have been caused by the effect of heat on some of the complex carbohydrates making up the seed coat component. Heat can break down some complex carbohydrates to less complex ones (Jobling 1983). The same phenomenon may also have caused the significantly increased level of NFE observed in MSM subjected to boiling (Table 2).

The very high level of digestibility as shown in the ADC protein for the control diet (93.33%) that is significantly differently from the other diets (40.7 %-56.19 %) (Table 3) may not be unconnected with the simple ingredient (casein) used as protein source. This is in contrast with the complex protein found in MSM. Hybrid catfish used in this study may have found it easier to digest simple proteins but difficult to digest complex ones. This partly explains the lower ADC protein values obtained with MSM diets. However, processing by dehulling seems to have slightly improved though not significantly the ADC protein of the raw MSM from 40.17 % to 46.25 %. Boiling the undehulled raw MSM also produced same effect by slightly improving the ADC protein. On the other hand, boiling combined with dehulling produced ADC protein for the MSM that is significantly higher than the values for undehulled boiled and dehulled raw MSM.

Seeds of Mucuna species possess anti nutritional factors such as phenols, phytates, saponins, lectins, protease inhibitors, L-DOPA etc (Table 4) like other legume seeds (Siddhuraju and Becker 2001; Adebowale *et al*. 2005). These anti nutritional factors interfere negatively on protein digestion in fish in various ways. Phytates can form phytic-protein complexes which reduce the availability of dietary protein (Richardson *et al.* 1985). Endogenous saponins can reduce the protein digestibility of soybean by chymotrypsin (Shimoyamada *et al*., 1998) through the formation of sparingly digestible saponin protein complexes (Potter *et al*. 1993). Lectins resist proteolytic breakdown leaving substantial quantities of ingested lectin in the digestive tract and faeces (Nakata and Kimura 1985, 1986; Oliveira *et al*.1994). Fish is sensitive to protease inhibitors and a direct relationship is observed between the amount of trypsin inhibitor in the diet and availability of protein and energy for trout (Sandholm et al., 1976; Krogdahl et al. 1994). Oligosaccharides and non starch polysaccharides present in MSM can lower feed digestibility in trout (Sanz et al. 1994). Tannins exert their anti nutritional effects by interfering with the digestive process by binding the enzymes or binding to feed components like protein (Liener 1989). Carnovale *et al.* (1991) reported the formation of insoluble tannin – protein complexes which leads to decreased protein digestion. L-DOPA, a non protein phenolic amino acid is potentially toxic to monogastrics and other animals (Duke 1981). The oxidation product of L-DOPA conjugates with S-H compounds (cysteine) of protein to form protein bound 5-S-cysteinyl-dopa crosslinks which leads to polymeration of protein (Takashi and Kawakishi 1979). However, common cultured fish differ in their ability to tolerate dietary anti-nutritional factors (Olli *et al.* 1994; Robaina *et al.* 1995; Wee and Shu 1989; Makka and Becker 1999).

Some of these anti-nutritional factors are concentrated in the outer layers (coat) of the seed. The slight improvement in the ADC protein of dehulled raw diet (Table 4) over the undehulled raw diet corroborates the fact that dehulling removed anti nutritional factors located in the seed coat. This is in line with the recommendation of dehulling as a measure for removing anti nutritional factors in seed legumes (Griffiths 1991; Francis *et al.* 2001). Also

Siddhuraju and Becker (2001) had reported that dehulling of Mucuna seed coat substantially removed its total phenol constituent. The insignificant improvement in ADC protein of dehulled raw MSM suggests that the cotyledon contains considerable quantities of anti nutritional factors which exerted profound negative effect on protein digestion of the hybrid catfish.

Moist heat is effective in destroying or reducing antinutritional factors such as protease inhibitor activities, lectin activity, total phenols, tannins, phytates and L-DOPA in Mucuna (Siddhuraju and Becker 2001). In spite of this ability, boiling undehulled MSM only caused a slight improvement in the ADC protein. This may not be unconnected with the presence of the seed coat whose higher crude fibre content inhibits digestibility. Fagbenro (1999) reported an inverse relationship between apparent digestibility of nutrients in aquatic animals and fibre contents. Moreover, the presence of antinutrients in the seed coat results in higher percentage of whole seed protein being complexed with the anti nutritional factors rendering them unavailable for digestion with its concomitant reduced digestibility.

The enhanced performance of ADC crude protein of dehulled boiled MSM relative to undehulled boiled MSM may be caused by either of two facors: the absence of the high crude fibre seed coat with its antinutritional factor content, which made the heat treatment to be more effective in the removal of the heat labile anti nutritional factors in the cotyledon or the heat stable anti nutrients responsible for reduced protein digestion are predominant in the seed coat.

Boiling at the level employed in this study may have also had some negative impact on the quality of MSM protein. Viola *et al*. (1983) had earlier reported that heat treatment can destroy some essential amino acids (EAAs) in some legumes. Though it has been shown that Mucuna seed contains adequate lysine and deficient in methionine/cysteine (Adebowale *et al*. 2005), boiling may have led to further loss of heat sensitive EEAs. This may have been responsible for the relative low ADC crude protein of 56% recorded in this study.

However, the highest ADC crude protein value of 56% obtained from boiled dehulled MSM in this study is lower than what is obtained in many oil seed cakes/legume seeds for African catfish. Fagbenro (1998) reported generally high ADC crude protein of 78.3%-86.9% for oil seed cakes for *C.gariepinus*. Brown *et al*. (1985) had ADC crude protein of 85% for soybean meal for Channel catfish while Fagbenro, (1996) reported ADC crude protein of 85% for soybean for dwarf catfish. The ADC crude protein of 76.6% and 75% for cotton seed cake and peanut cake respectively reported by Fagbenro (1996) for dwarf catfish were also higher than the value obtained in this study for hybrid catfish. Improving the biological value of MSM protein by supplementing with the deficient amino acids may likely improve the ADC crude protein of dehulled boiled MSM for hybrid catfish to levels obtained in other seed legumes for other African catfish species.

MSM used in this study contained carbohydrates ranging from approximately 51% for raw MSM toabout 54% for the boiled MSM (Table 1). These values are higher than about 47% reported for *M.cochinchinensis* by Adebowale *et al*. (2005). Heat treatment is known to breakdown long chain carbohydrates making them more digestible (Bangoula, *et al*. 1993; Boujard *et al*. 2000)

The low values of ADC gross energy with raw MSM (Table 4) in this study may have been due to the presence of some indigestible complex carbohydrates and other antinutrients. Adebowale *et al*. (2005) reported the presence of raffinose, starchyose and verbascose carbohydrate antinutrients in *M.cochinchinensis* seeds while Siddhuraju and Becker (2001) reported that Mucuna contains 11% non starch polysaccharides (NSP). The presence of soluble NSP in fish diet reduces the availability of nutrients especially fat and reduces lipolysis (Svihus *et al*. 1997; Pasquier, et al. 1996). Consequently, the poor utilization of fat and carbohydrate as well as protein may have led to the low values of ADC gross energy observed in raw MSM.

The value obtained from dehulled boiled MSM (79.5%) was highest though similar to ADC gross energy for the control diet which had similar values as undehulled boiled MSM (63.09%). This shows

Table 4: Antinutritional factors in some raw and autoclaved Mucuna seed species ($g\ 100g^{-1}$ dry matter unless otherwise stated)

	Raw *M. cochinchinensis*[1]	Raw *M.pruriens*[2]	Autoclaved *M.pruriens*[2]
Total phenols	6.53	5.54	2.26
Tannins	-	0.37	0.20
Phytates	1.50	0.90	0.48
Saponins	1.46	1.15	0.51
L-DOPA	4.99	4.70	1.85
Trpsin inhibitor [a]	23.60	13.78	0.55
Chymotrypsin inhibitor	-	10.97	BLD
Phytoagglutinin activity [c]	-	0.20	BLD
Raffinose	1.45	-	-
Starchyose	1.05	-	-
Verbacose	1.03	-	-

L-DOPA – 3,4-dihydroxyphenylalanine
BLD - Below level of detection
[a] mg pure trypsin inhibited/g sample
[b] CIU chymotrypsin inhibitor unit/mg sample
[c] Phytohaemagglutinating unit (IIU/mg sample)
[1] Adebowale *et al.* (2005)
[2] Siddhuraju and Becker (2001)

that boiling of the MSM produced significantly improved performance on the ADC gross energy. Apart from making carbohydrates more digestible, heat treatment can destroy some of the anti nutrients that inhibit digestion of carbohydrates (De la Higuera 1988). Fagbenro (1998) obtained ADC gross energy of 79.2% with sunflower cake for *C.gariepinus* that is similar to 79.5% with dehulled boiled MSM for hybrid catfish obtained in this study. Slightly lower values of (77.4% and 75.6%) for soybean cake and peanut cake respectively were however obtained using *C.gariepinus*. Thus hybrid catfish has a high ADC gross energy for *M. cochinchinensis* as obtained in other catfishes for oilseeds.

The apparently better performance of dehulled boiled MSM with ADC gross energy of 79.5% over the control diet (69.20%) may have to do with better utilization of lipids in boiled MSM since lipid contributes substantially to the total energy values.

It is concluded from the results obtained that though dehulling combined with boiling made MSM to have ADC values that are better than other forms of processing used in this study, measures to further improve particularly the ADC protein should be adopted in order to attain the status of being a suitable fishmeal substitute in hybrid catfish diets. This probably can be achieved by improving the biological value of MSM by amino acid supplementation given that MSM is deficient in methionine.

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