Evaluation of Ground - Sundried Cassava Leaf Meal as Protein Source for Nile Tilapia *Oreochromis niloticus* (L) Juvenile's Diet

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Abstract

Ground and sun dried cassava leaf meal (CLM) was evaluated as a protein source for Oreochromis niloticus juveniles. Biochemical analyses, digestibility study and growth trial were undertaken. Biochemical analyses showed that grinding followed by sun drying resulted into 60% reduction of hydrogen cyanide while saponins, phenols, tannins and phytic acid were slightly affected. Crude lipid and crude fibre contents were also reduced. Digestibility trial showed that CLM has low apparent digestibility of protein (44.5%) and energy (44.2%). Increased inclusion of CLM from 0, 15, 30, 45 to 60 g100 g-1 of total dietary crude protein resulted into a significant reduction of growth, nutrient utilisation and whole body composition after an eight week growth trial. SGR declined from 3.17% in fish fed CLM0 to 1.15% in those fed CLM60. Similarly, FCR increased from 1.45 in fish fed CLM0 to 5.02 in those fed CLM60. Whole body lipid declined from 8.29% fed CLM0 to 2.61% in those fed CLM60. Histopathological examination of liver and small intestine did not show any changes that could be linked to dietary treatment. Therefore, there is need to improve digestibility of CLM in order to realise its potential as a protein source in Nile tilapia diets.

Key words: Nile tilapia, cassava leaf meal, digestibility, nutrient utilisation

Introduction

Cassava, *Manihot esculenta* Crantz is a multipurpose perennial woody shrub with edible leaves and roots. It is a member of the family *Euphorbiaceae* with origins in Latin America, primarily grown for its starchy roots. Leaves have relatively high crude protein which varies from 17.8% to 34.8% with an average of 25% of which almost 85% is true protein (Smith, 1992; Ravindran, 1992). The amino acid profile of cassava leaves compares well with that of soybean meal except that they are deficient in sulphur containing amino acids (Eggum, 1970). Cassava leaves are also good sources of vitamins such as ascorbic acid, vitamin A and riboflavin as well as minerals like iron, manganese and zinc (Ravindran, 1992). Leaf yields of up to 10 tonnes dry matter per hectare may be harvested without adversely affecting root production (Khajarern and Khajaren, 1992).

Despite their nutritional merit, cassava leaves have high content of anti-nutritional factors (hydrogen cyanide and tannins) and fibre (Ravindran *et al.* 1987a). Hydrogen cyanide is the most significant antinutritional factor in cassava leaves whose acute toxicity leads to sudden death while less acute toxicity may cause gastrointestinal disorders and reduced growth.

Higher inclusion levels of unprocessed cassava leaves has been reported to result in reduced growth in Nile tilapia, *Oreochromis niloticus* (Ng and Wee, 1989), low digestibility in Asian sea bass, *Lates calcarifer* (Eusebio and Coloso, 2000) and increased susceptibility to diseases in African catfish, *Clarias gariepinus* (Bureau *et al.,* 1995).

Despite poor performance, cassava leaves still have a potential to serve as a cheap source of protein in fish feeds in Tanzania due to their

abundance in supply. Tanzania is among the top five producers of cassava in Africa, which is primarily grown for its starchy roots and it is estimated that about 7 million metric tonnes are produced annually (Lekule and Sarwatt, 1992). A large proportion of the cassava leaves go to waste as a by–product of cassava root production. Lack of affordable and easily available protein sources is one of limiting factors hindering tilapia farming in Tanzania. Fish meal which is most preferred source of protein is unaffordable due to its use in terrestrial livestock feed industry and as human food. Therefore this study evaluated suitability of ground - sun dried cassava leaf meal as a protein source in diets for Nile tilapia. It is hypothesised that inclusion of cassava leaf meal in the diets will have no significant effect on performance.

Materials and methods

Study site and experimental facilities

The digestibility and growth trials were conducted in a recirculation system within the tropical aquarium facility of the Institute of Aquaculture, University of Stirling, Scotland.

Processing of cassava *M. esculenta* leaves

Fresh mature cassava leaves from a variety locally known as "betauje" were harvested during the dry season from the slopes of the Uluguru Mountains in Morogoro region, Tanzania. The harvested leaves were manually stripped of petioles to reduce crude fibre content. This was followed by grinding using a traditional wooden mortar and pestle followed by sun drying for 24 hours on plastic sheets to remove hydrocyanic acid. The dried leaf meal was ground into a fine powder using a hammer mill (Lab Mill, screen size 0.2 mm) and then stored in a plastic bag in a dry place at room temperature.

Biochemical analysis

Biochemical analyses were conducted on cassava leaf meal and formulated diets to determine their biochemical composition using standard methods (AOAC, 1990). Gross energy was quantified using adiabatic bomb calorimeter (Parr 6100, Illinois, USA) with benzoic acid serving as a standard. Amino acid content was quantified using LKB Biochrom 4151 Alpha plus amino acid analyser (LKB Biochrom 30+, Cambridge, UK). Phosphorus was analysed following a procedure of Allen (1989) while sodium, potassium, calcium, iron, zinc, manganese, magnesium and copper were quantified using a Thermo X series 2 Inductively Coupled Plasma Mass Spectrophotometer (ICP MS) (Thermo Scientific, Massachusetts, USA). Anti-nutritional factors i.e. total saponins, hydrogen cyanide (HCN) and tannins were determined according to the method described by Baccou *et al.* (1977), Bradbury *et al.* (1999) and Allen (1989), respectively, while phytic acid was determined using an assay kit (Megazyme, K-Phyt 05/07, Wicklow, Ireland).

Fish handling and digestibility trial

The Nile tilapia fingerlings used in this study were of the red Stirling strain. Fish handling was conducted according to United Kingdom Home Office regulations as stipulated by The Animals (Scientific Procedures) Act 1986. Apparent digestibility coefficients of processed cassava leaf meal (CLM) were determined using test and reference diets formulated according to recommendations by Cho *et al,* (1982) as shown in Table 1.

Table 1: Formulation of diets used to determine apparent digestibility coefficients of processed cassava leaf meal (g 100 g^{-1})

Ingredient	Reference Diet	Test diet
Processed cassava leaf meal[1]	0.00	29.85
Fishmeal[2]	30.00	21.00
Soybean meal[3]	8.10	5.67
Wheat meal[4]	47.20	33.04
Sunflower oil	6.20	4.34
Vitamin premix[5]	2.00	1.40
Mineral premix[6]	4.00	2.80
CMC[7]	2.00	1.40
Chromium (III) oxide	0.50	0.50

[1]aqueous extracted, [2]brown fishmeal (aquaculture grade), [3]dehulled, solvent extracted, [4]whole grain, [5]Contained (as g.kg^{-1} of diet): $MgSO_4.7H_2O$, 20.40; NaCl, 8.00; KCl, 6.04; Fe $SO_4.7H_2O$, 4.00; $ZnSO_4.4H_2O$, 0.88; $MnSO_4.4H_2O$, 0.41; $CuSO_4.5H_2O$, 0.13; $CoSO_4.7H_2O$, 0.08; CaIo3,$6H_2O$,

0.05; $CrCl_3.6H_2O$, 0.02 (according to Jauncey and Ross 1982). 6Contained (as mg.kg^{-1} of diet): Thiamine (B_1), 85.00; Riboflavin (B_2), 60.00; Pyridoxine (B_6), 25.00; Pantothenic acid, 105.00; Inositol, 500.00; Biotin, 1.80; Folic acid, 20.00; Ethoxyquin, 4.00; Choline, 1481.00; Nicotinic acid (Niacin), 250.00; Cyanocobalamin (B_{12}), 0.03; Retinol palmitate(A), 20.00; Tocopherol acetate (E), 140.00; Ascorbic acid (C), 750.00; Menadione (K), 30.00; Cholecalciferol (D_3), 0.08 (according to Jauncey and Ross 1982). 7Carboxymethylcellulose (sodium salt, high viscosity)

Chromium (III) oxide (BDH 277574Q) was used as an inert marker at an inclusion level of 0.5%. A total of 45 fish with an average weight (± SD) of 13.1 ± 1.89 g were group-fed fish to apparent satiation twice a day. Fish faeces were collected using a modified Guelph system as described by (Cho *et al.,* 1985).

Apparent digestibility coefficients (ADC) of the reference and test diets were computed using the formula described by Maynard and Loosli (1969):

$$\text{ADC (\%)} = 100 \times \left(\frac{\text{\% nutrient in faeces}}{\text{\% nutrient in feed}}\right) \times \left(\frac{\text{\% marker in feed}}{\text{\% marker in faeces}}\right)$$

Apparent digestibility coefficients for CLM were estimated according to Lupatsch (2003) using the following formula:

$$DC_T = \frac{\left[DC_D - \left(DC_R \times \{1\text{-}t\}\right)\right]}{t}$$

Where:

DCT = Digestibility coefficient of the nutrient in test ingredient (%)

DCD = Digestibility coefficient of the nutrient in whole diet (%)

DCR = Digestibility coefficient of the nutrient in reference diet (%)

t = Contribution of nutrient of test ingredient to total diet; calculated as

$$100 - \frac{\textit{nutrient concentration in R} \times \textit{inclusion of R in D\%}}{\textit{nutrient concentration in DD}}$$

where R=reference ingredient, T=test ingredient and D=R+T, whole diet.

Digestible protein (DP) and digestible energy (DE) were calculated as follows:

Where:

ADCP = Apparent Digestibility Coefficient for Protein

ADCE = Apparent Digestibility Coefficient for Energy

CP = Crude protein content

GE = Gross energy content

Growth trial

Five diets i.e. CLM0, CLM15, CLM30, CLM45 and CLM60 were formulated in which CLM provided 0, 15, 30, 45 and 60 g 100 g^{-1} of total dietary crude protein respectively. Diet CLM0 served as control and contained fishmeal as a main source of protein. All diets were formulated to contain 30 g 100 g^{-1} crude protein, 18 kJ g^{-1} and 10 g 100 g^{-1} lipid (Table 2).

Table 2: Formulation of diets fed to *O. niloticus* during the growth trial for cassava leaf meal (g 100 g^{-1}, as fed)

Ingredients	CLM 0	CLM 15	CLM 30	CLM 45	CLM 60
Fishmeal[1]	36.5	31.5	26.0	21.0	16.0
Cassava leaf meal	0.0	15.5	31.5	48.5	62.5
Wheat meal[2]	43.5	34.0	24.5	14.0	6.0
Sunflower oil	6.0	6.5	6.5	7.0	7.0
Vitamin premix[3]	2.0	2.0	2.0	2.0	2.0
Mineral premix[4]	4.0	4.0	4.0	4.0	4.0
CMC[5]	2.0	2.0	2.0	2.0	2.0
α-cellulose	5.5	4.0	3.0	1.0	0.0
Chromium oxide	0.5	0.5	0.5	0.5	0.5

[1]Brown fishmeal (aquaculture grade), [2]Whole grain, [3]As listed under Table 1, [4]As listed under Table 1, [5]Carboxymethylcellulose

Twenty Nile tilapia fingerlings with an average weight (±SD) of 3.86 ± 0.31 g were stocked into each of the 15 self-cleaning circular plastic tanks with a capacity of 30 litres and fed trout diet (Nutra Trout Fry 02, Skretting UK) during acclimatization for one week. After acclimatization dietary treatments were randomly assigned to the 15 culture tanks in

triplicates. Fish were bulk weighed once every week during the eight week growth trial period. The diets were fed daily to apparent appetite in three equal rations at around 09:00 hrs, 13:00 hrs and 17:00 hrs. Final body weights were recorded at the end of the trial. Likewise, five fish were randomly sampled from each tank for whole body proximate composition and histopathology analyses. The rest of the fish from each replicate were pooled according to their dietary treatments for faecal collection to determine apparent digestibility of the diets.

Performance variables

Performance variables were determined in terms of feed intake (FI), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilisation (ANPU), energy retention (ER), hepatosomatic index (HSI) and digestible energy to digestible protein ratio (DE/DP) as follows:

$$FI(g\ fish^{-1}\ day^{-1}) = \frac{total\ feed\ \text{intake}\ per\ fish}{number\ of\ days}$$

$$ADG(g\ fish^{-1}\ \text{day}^{-1}) = \frac{final\ weight - initial\ weight}{number\ of\ days}$$

$$FCR = \frac{\text{feed intake}}{live\ weight\ gain}$$

$$PER = \frac{live\ weight\ gain}{crude\ protein\ \text{intake}}$$

$$ANPU(\%) = 100 \times \frac{\{[final\ fish\ body\ protein] - [final\ fish\ body\ protein]\}}{crude\ protein\ \text{intake}}$$

$$ER(\%) = 100 \times \frac{\{[final\ fish\ body\ energy] - [final\ fish\ body\ energy]\}}{crude\ protein\ \text{intake}}$$

$$HSI = 100 \times \frac{liver\ weight}{body\ weight}$$

$$DP/DE\ ratio(mg\ DP/kJ\ DE) = \frac{Digestible\ protein}{Digestible\ energy}$$

Whole body samples were analysed for proximate composition (AOAC, 1990)and results expressed as percentage of live weight.

Histopathology of gut and liver

Histological analyses of liver and gut were carried out to detect any pathological changes due to dietary treatments. The samples were fixed in 10% neutral buffered formalin. The tissues were examined as described by Drury and Wallington (1980).

Data analysis

Performance variables among the different dietary treatments were analysed using one way analysis of variance (ANOVA) to determine differences between means. Tukey's Honest Significant Difference Test was done in case of significant differences. Before ANOVA, the data were tested for normality and homogeneity of variance using the Kolmogorov–Smirnov test and Levene's test respectively. Percentage data were arcsine transformed before performing ANOVA. Results are presented as mean ± SE and were considered statistically significant at $P \leq 0.05$ (Ott and Longnecker, 2001). The analyses were performed using SPSS software version 13 (SPSS Inc.)

Results

Biochemical composition and digestibility

The biochemical composition of processed and unprocessed cassava leaf meal is shown in Table 3 to Table 5. Grinding followed by sun drying of cassava leaf meal decreased crude lipid by about 53% and crude fibre by about 22% (Table 3). There was a slight increase in crude protein and ash which was reflected by the increased content of some amino acids (Table 4) and minerals (Table 5) respectively. Processing was fairly effective in removing about 60% of the hydrogen cyanide. Similarly, the contents of other antinutritional factors i.e. saponins, phenols and tannins were also reduced but that of phytic acid increased.

Table 3: Proximate composition (g 100^{-1} g, as fed) and Gross energy (kJ g^{-1}) of unprocessed and processed CLM

Item	Unprocessed CLM	Processed CLM
Dry matter	95.94	93.36
Crude protein	28.79	29.00
Crude lipid	5.02	2.37
Crude fibre	13.01	10.21
Ash	5.64	5.95
Nitrogen free extract	43.51	45.84
Gross energy (kJ g-1)	20.58	19.66

Table 4: Amino acid content (g $100g^{-1}$ feed) of unprocessed and processed CLM

Amino Acid	Unprocessed CLM	Processed CLM
Arginine	1.53	1.77
Histidine	0.73	0.77
Isoleucine	1.54	1.62
Leucine	2.44	2.67
Lysine	1.58	1.49
Methionine + Cystine	0.47	0.47
Phenylalanine+ Tryptophan	2.73	2.81
Threonine	1.33	1.27
Valine	1.89	1.99

Table 5: Mineral content (mg g^{-1}, DM) in unprocessed and processed CLM

Mineral	Unprocessed CLM	Processed CLM
Phosphorus	4.06	4.11
Sodium	0.21	0.18
Magnesium	2.54	2.45
Potassium	12.44	14.45
Calcium	5.98	6.29
Iron	0.12	0.15
Copper	0.02	0.01
Zinc	0.07	0.06

Table 6: Antinutritional factors content (g 100 g^{-1}, DM) in unprocessed and processed CLM

Antinutritional factors	Unprocessed CLM	Processed CLM
Hydrocyanic acid[1]	4.34	1.74
Saponins[2]	1.33	1.06
Phenols[2]	5.43	4.94
Tanins[3]	2.70	2.12
Phytic acid[4]	0.19	0.24

[1]mg $100g^{-1}$, [2]As diosgenin equivalent, [3]As tannic acid equivalent, [4]As phosphorus equivalent

Proximate composition of test and reference diets used to determine digestibility coefficients of processed CLM is shown in Table 7.

Table 7: Proximate composition (g 100 g^{-1}, as fed) of test and reference diets used to determine digestibility coefficients of processed cassava leaf meal (CLM)

Item	Reference Diet	Test diet
Dry matter	93.08	94.81
Crude protein	31.56	31.35
Crude lipid	10.69	8.62
Crude fibre	2.81	4.85
Ash	8.66	8.18
Nitrogen free extract	39.36	41.81
Gross energy (kJ g^{-1})	17.92	19.22

Results from the digestibility trial showed that CLM had low digestibility. With exception of crude lipid, whose apparent digestibility coefficients (ADC) were above 70%, the ADC values for other components were less than 50% (Table 8).

Table 8: Apparent digestibility coefficient (%) of processed cassava leaf meal (CLM)

Item	Reference Diet	Test diet	CLM
Dry matter	73.3	62.7	38.3
Crude protein	84.3	72.4	44.5
Crude lipid	98.4	95.8	88.3
Gross energy	70.1	61.9	44.2
Digestible protein (g $100g^{-1}$)	24.8	22.4	12.7
Digestible energy (kJ g^{-1})	12.6	11.9	9.2

Table 9: Proximate composition (g $100g^{-1}$, as fed) of CLM diets used for the growth trial

Item	CLM 0	CLM 15	CLM 30	CLM 45	CLM 60
Dry matter	95.02	95.49	95.3	94.58	95.13
Crude protein	32.38	31.42	32.02	31.51	31.33
Crude lipid	11.30	11.05	11.23	11.42	11.44
Crude fibre	5.98	6.06	6.88	7.51	8.06
Ash	9.42	9.46	9.64	9.61	9.69
Nitrogen free extract	35.94	37.5	35.53	34.53	34.61
Gross energy (kJ g^{-1})	18.39	18.89	19.12	19.95	20.43
Phosphorus (mg g^{-1})	9.22	7.84	7.73	7.58	6.59

Prcoximate analysis of the CLM diets showed that the diets had similar contents of crude protein, crude lipid and ash irrespective of CLM inclusion level (Table 9). However, there was an increase in crude fibre and gross energy content as CLM inclusion level increased while that of phosphorus declined. The CLM diets met the essential amino acid requirements of Nile tilapia with the exception of diets CLM45 and CLM60 which were deficient in methionine + cystine (Table 10). Levels of antinutritional factors in the growth trial diets increased with increasing CLM inclusion level with contents of phenols, tannins and saponins being greatest (Table 11).

Table 10: Amino acids (g $100g^{-1}$ feed) content of CLM diets used for the growth trial

Amino acids	CLM0	CLM15	CLM30	CLM45	CLM60	Req*
Arginine	6.09	6.11	6.13	6.16	6.17	4.20
Histidine	2.36	2.40	2.45	2.49	2.53	1.72
Isoleucine	4.27	4.48	4.69	4.91	5.09	3.11
Leucine	7.99	8.12	8.26	8.39	8.52	3.39
Lysine	7.07	6.84	6.58	6.34	6.10	5.12
Methionine + Cystine	3.96	3.61	3.25	2.87	2.55	3.21
Phenylalanine + Tryrosine	7.05	7.43	7.83	8.23	8.59	5.54
Valine	4.00	4.06	4.12	4.18	4.23	2.80

**Req = O. niloticus amino acid requirements (Santiago and Lovell, 1988)*

Table 11: Antinutritional factors (g $100g^{-1}$ feed) content of CLM diets used for the growth trial

Amino acids	CLM 0	CLM 15	CLM 30	CLM 45	CLM 60
Hydrogen cyanide[1]	-	0.27	0.55	0.84	1.09
Phenols[2]	-	0.77	1.56	2.40	3.09
Tannins[2]	-	0.33	0.67	1.03	1.33
Saponins[3]	-	0.16	0.33	0.51	0.66
Phytic acid[4]	-	0.04	0.08	0.12	0.15

[1]Hydrocyanic acid (mg $100g^{-1}$), [2]As tannic acid equivalent, [1]As diosgenin equivalent, [4]As phosphorus equivalent

With the exception of lipid, the digestibility of other nutrients declined sharply with increasing CLM inclusion reflecting its poor digestibility (Table 12). There was a noticeable decline in digestible protein in comparison to that of digestible energy, resulting in a decline in DP/DE ratio with increased CLM inclusion level.

Table 12: Apparent digestibility coefficient (%) content of CLM diets used for the growth trial

Apparent digestibility coefficient (%)	CLM0	CLM15	CLM30	CLM45	CLM60
Dry matter (%)	75.8	71.0	65.9	61.4	58.9
Crude protein (%)	90.7	81.8	75.7	69.1	65.1
Crude lipid (%)	94.7	90.4	88.9	87.1	88.4
Gross energy (%)	75.5	70.6	65.8	62.2	60.4
Phosphorus (%)	80.4	78.1	78.4	75.1	71.9
Digestible protein (DP) (g $100g^{-1}$)	29.4	25.7	24.2	21.8	20.4
Digestible energy (DE) (kJ g^{-1})	13.9	13.3	12.6	12.4	12.3
DP/DE ratio (mg kJ^{-1})	22.3	20.2	20.2	18.6	17.4

Growth performance and nutrient utilisation
Fish fed CLM diets gained weight during the trial but the growth decreased with increasing inclusion of levels of CLM (Figure 1).

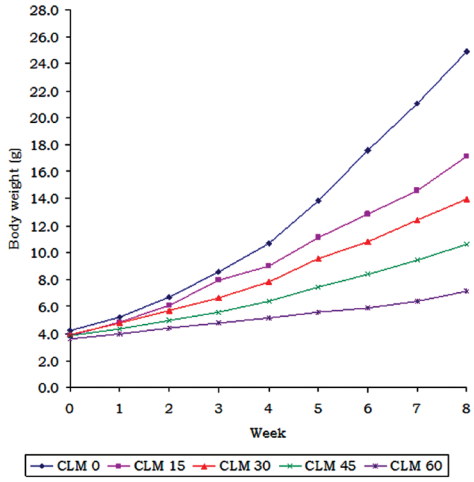

Figure 1: Change in body weight of *O. niloticus* fed diets containing different levels of processed cassava leaf meal (CLM)

Growth performance in terms of final weight, average daily weight gain and specific growth rate declined significantly with increasing CLM inclusion level (Table 13). For instance, SGR declined from 3.7 to 1.15 % day^{-1}. Fish did not show obvious signs of feed rejection, but feed intake declined with increasing CLM inclusion although not significant in some cases. Digestible energy and digestible protein intakes followed a similar trend. Inclusion of CLM resulted in a significant increase in feed conversion ratio from 1.45 to 5.02 indicating its poor utilization. Protein efficiency ratio, apparent net protein utilization, energy retention and hepatosomatic index also declined significantly with increasing CLM inclusion level. There was, however, no significant difference in fish survival between different dietary treatments.

Whole body composition
Initial and final whole body proximate composition is shown in Table 14. Final body moisture and ash contents were significantly higher in fish fed diet CLM60 while lipid and gross energy content was significantly lower. Crude protein contents were not significantly different.

Liver and gut histopathology
Liver and gut tissues did not show any pathology that could be linked to dietary treatment. However, liver tissues from fish fed diets containing higher levels of CLM (30 – 60g 100 g^{-1}) had less lipid deposition.

Discussion
The study aimed to evaluate the suitability of ground - sun dried cassava leaf meal as a protein source in diets for Nile tilapia. Grinding of cassava leaves followed by sun drying did not cause any significant change in protein or amino acid contents. This finding corroborates earlier reports that processing has little influence on CLM crude protein content (Ravindran, 1985). Crude protein contents of CLM both raw (28.79%) and processed (29.0%) were higher

than the reported average of 25% (Smith, 1992; Ravindran, 1992). The content of some amino acids and minerals increased slightly to reflect the slight increase in crude protein and ash contents respectively. However, in CLM diets there was gradual decline in essential amino acid content as CLM inclusion level increased causing a deficiency of sulphur amino acids in diets CLM45 and CLM60. Similar findings were reported by (Santiago and Lovell, 1988).

Table 13: Growth performance and nutrient utilisation of *O. niloticus* fed CLM diets (mean ± SE, n=3)

	CLM0	CLM15	CLM30	CLM45	CLM60
Initial weight (g)	4.22 ± 0.04^{a}	3.86 ± 0.14^{a}	3.93 ± 0.04^{a}	3.70 ± 0.27^{a}	3.61 ± 0.16^{a}
Final weight (g)	24.96 ± 085^{a}	17.45 ± 0.70^{b}	13.97 ± 0.32^{c}	10.57 ± 0.66^{d}	6.90 ± 0.31^{e}
Feed intake (g fish^{-1} day^{-1})	0.53 ± 0.02^{a}	0.45 ± 0.01^{ab}	0.40 ± 0.01b^{c}	0.35 ± 0.02^{c}	0.29 ± 0.01^{d}
DP intake(g fish^{-1} day^{-1})	0.157 ± 0.008^{a}	0.110 ± 0.003^{b}	0.097 ± 0.001b^{c}	0.076 ± 0.004^{cd}	0.060 ± 0.001^{d}
DE intake*(kJ fish^{-1} day^{-1})	7.43 ± 0.35^{a}	5.98 ± 0.17^{b}	5.08 ± 0.06b^{c}	4.32 ± 0.24^{c}	3.61 ± 0.07^{c}
Average daily gain (g fish^{-1} day^{-1})	0.37 ± 0.02^{a}	0.24 ± 0.01^{b}	0.18 ± 0.01^{c}	0.12 ± 0.01^{d}	0.06 ± 0.01^{e}
Specific growth rate (% day^{-1})	3.17 ± 0.05^{a}	2.69 ± 0.05^{b}	2.26 ± 0.05^{c}	1.88 ± 0.12^{d}	1.15 ± 0.05^{e}
Feed conversion ratio	1.45 ± 0.07^{a}	1.85 ± 0.03^{b}	2.26 ± 0.08^{c}	2.85 ± 0.10^{d}	5.02 ± 0.25^{e}
Protein efficiency ratio	2.14 ± 0.10^{a}	1.72 ± 0.03^{b}	1.39 ± 0.05^{cd}	1.12 ± 0.04b^{c}	0.64 ± 0.03^{d}
Apparent net protein utilization (%)	35.94 ± 2.17^{a}	27.41 ± 0.59^{b}	21.54 ± 1.44	18.55 ± 0.59^{c}	10.16 ± 1.18^{d}
Energy retention (%)	19.28 ± 0.65^{a}	15.02 ± 0.55^{b}	12.73 ± 0.20^{b}	9.30 ± 0.39^{c}	4.14 ± 0.13^{d}
Hepatosomatic index	3.41 ± 0.48^{a}	2.87 ± 0.36^{ab}	2.57 ± 0.27^{b}	2.41 ± 0.24^{b}	1.65 ± 0.27^{c}
Survival (%).	95.00 ± 5.00^{a}	96.67 ± 3.33^{a}	100.00 ± 0.00^{a}	98.33 ± 1.67^{a}	98.33 ± 1.67^{a}

Different superscripts in the same row indicate significant difference ($p<0.05$)

The removal of almost 60% of the HCN content demonstrate the effectiveness of grinding and sun drying of cassava leaves. Similar finding was reported earlier (Ravindran *et al.,* 1987; Ravindran, 1992; Phuc *et al.,* 2000; Fasuyi, 2005). Grinding disrupts cellular structure and facilitates a reaction between cyanogenic glycosides (linamarin and lotaustralin) and the enzyme linamarase which are stored separately in plant cells (Oke, 1978). This facilitates hydrolysis of cyanogenic glycosides into HCN which is then volatilised during sun drying. The contents of other antinutritional factors like saponins, phenols and tannins were also reduced after processing. Reduction of phenols

The reduction in crude fibre content was due to removal of petioles during processing. Ravindran (1985) reported a 17% reduction after a similar procedure with mature cassava leaves. The crude fibre content, however, still remained high (10 g 100 g^{-1}) and consequently fibre content in diet CLM60 (8 g 100 g^{-1}) was higher than the recommended level of 5 g 100 g^{-1} (Anderson *et al.* 1984; Ali *et al.* 2003; Dioundick and Stom, 1990).

Table 14: Whole body proximate composition of *O. niloticus* fed CLM diets before and after the experiment (% fresh weight basis, mean ± SE, n=3)

Item	Before	After				
		CLM0	CLM15	CLM30	CLM45	CLM60
Moisture	76.04	70.99 ± 0.50^{a}	71.60 ± 0.62^{a}	73.01 ± 0.73^{a}	72.90 ± 0.96^{a}	77.62 ± 1.14^{b}
Crude protein	13.64	16.23 ± 0.25	15.41 ± 0.38	14.98 ± 0.40	15.59 ± 0.58	14.72 ± 0.72
Crude lipid	6.22	8.29 ± 0.14^{a}	8.35 ± 0.16^{a}	7.37 ± 0.23^{b}	6.67 ± 0.21^{b}	2.61 ± 0.13^{c}
Ash	3.01	3.03 ± 0.012^{a}	3.31 ± 0.075^{b}	3.43 ± 0.012^{b}	3.59 ± 0.017^{c}	4.52 ± 0.012^{d}
Gross energy (kJ g^{-1})	5.26	5.34 ± 0.02^{a}	5.28 ± 0.03^{ab}	5.15 ± 0.02^{bc}	5.03 ± 0.03^{c}	4.26 ± 0.12^{d}

Different superscripts in the same row indicate significant difference ($p<0.05$)

and tannins in processed cassava leaves has been reported by Fasuyi (2005). Makkar and Singh (1993) suggested that such a reduction is possibly due to oxidation of tannins by polyphenol oxidase. However, the reduction was not substantial as that of HCN and according to Rickard (2006) residual tannins may be a major factor limiting the nutritional value of cassava leaf meal. The content of all antinutritional factors increased with increasing CLM inclusion level in the diets, hence, resulting in reduced feed intake, growth and nutrient utilisation (Mehansho *et al.* 1987). Sub-lethal doses of HCN remaining after processing are known to trigger detoxification processes which tend to increase the demand for methionine. According to Oke (1978), HCN is converted to thiocyanate within the body in the presence of the enzyme rhodanase using methionine as a sulphur donor. This process can potentially result in amino acid imbalance as the bioavailability of methionine in cassava leaves is naturally poor (Eggum, 1970).

Results from the digestibility trial showed that CLM is poorly digestible in terms of dry matter, crude protein and gross energy. The apparent protein digestibility coefficient (45%) was very low compared to the range of 75% to 95% suggested for dietary protein sources (NRC, (1993). Similarly the apparent gross energy digestibility had a low value (44.17%). Consequently diets with high CLM inclusion could not meet energy requirement of fish. Fish are generally known to compensate for low energy density in feed by eating more as long as the physical capacity of the digestive tract permits and the feed is sufficiently palatable (Cho and Bureau, 1995). Poor digestibility also affected DP/DE ratio, particularly for diet CLM60 whose value was below the 18 mg kJ^{-1} recommended for Nile tilapia (Kaushik *et al.* 1995).

Poor digestibility of cassava leaf meal is possibly due to a number of reasons. According to Ravindran (1993) only 85% of CLM protein is true protein and the remaining 15% is non-protein nitrogen (NPN) which tilapias are incapable of utilising (Viola and Zohar, 1984). Moreover, Reed *et al.* (1982) found that a large proportion of the protein in cassava leaves is bound to its crude fibre fraction thus making it unavailable to digestive enzymes. Furthermore, the bound protein is strongly associated with condensed tannins which are known to form indigestible protein-tannin complexes (Reed *et al.,* 1982). Thus, despite attempts to balance crude fibre content in CLM diets using α-cellulose, protein digestibility declined drastically exhibiting a 25% difference between diet CLM0 and diet CLM60 while crude fibre only varied by 2%. Moreover, the α-cellulose added to diet CLM0 (5.5 g 100 g^{-1}) was approximately within the range of 2.5 to 5.0 g 100 g^{-1}. Similar range has been reported to promote growth and feed conversion ratio in O. mossambicus (Dioundick and Stom, 1990). This suggests that it is quality rather than quantity of crude fibre which affected digestibility in CLM diets. However, the carbohydrate content of CLM is fibrous rather than starchy due to its fibre content hence contributing to poor digestibility. According to Bureau *et al.* (2002), the digestible

energy of such ingredients tends to be less than half of their gross energy content. Interestingly, in the current finding DE of 8.04 kJ g^{-1} was less than half of GE of 19.66 kJ g^{-1}. Similarly, Sklan *et al*. (2004) when fed tilapia with wheat bran containing 8.6% crude fibre observed poor DE of 6.95 kJ g^{-1} which was less than half of the GE of 17.94 kJ g^{-1}. Similar observations have been made for common carp fed diets whose carbohydrate fraction was rich in crude fibre that resulted in low energy digestibility (56%) (Kirchgessner *et al.,* (1986). The low body lipid content of fish fed diet CLM60, which paralleled a low hepatosomatic index, reaffirms poor energy intake. Fish tend to utilise lipid reserves to sustain metabolism when food energy is not sufficient, and this results in body lipid loss (Hepher, (1988). Poor growth observed in this study is similar findings reported by Ng and Wee (1989).

Results from this study have shown that grinding followed by sun drying was fairly effective in removing HCN from cassava leaves but not other antinutritional factors. CLM was poorly digestible and its inclusion in diets led to poor intakes of digestible energy and digestible protein which consequently resulted in poor overall performance. Further studies are recommended to explore means of improving CLM digestibility by reducing or degrading crude fibre and antinutritional factors, particularly tannins. The study recommends inclusion of ground and sun dried cassava leaf meal at less than 15 g 100 g^{-1} of dietary protein in Nile tilapia diets, the lowest level tested in the present study.

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