Evaluation of Aqueous Extracted Moringa Leaf Meal as a Protein Source for Nile Tilapia Juveniles

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Abstract

This study was conducted to evaluate suitability ofmoringa leaf meal (MLM) as an alternative source of protein for Oreochromis niloticus. MLM was soaked overnight in tap water at 1:1 w/v to remove saponins and other water soluble antinutritional factors (ANF). Soaking resulted in a decrease in MLM crude protein content from 34.9 to 31.1g 100-1g but no effect on saponins which remained at 1.2 g 100-1g. Digestibility trial showed that MLM had high digestibility for protein (89%) and energy (76.8%). Growth trial used five isonitrogenous (30g 100-1g), isolipidic (10g 100-1g) and isoenergetic (18 kJ g-1) diets in which MLM provided 15, 30, 45 and 60 g 100-1 of total dietary protein. This led to a significant decline ($p < 0.(5)$ in feed intake (0.71, 0.56, 0.50, 0.45 and 0.40g fish-1 day-1), specific growth rate (2.73,2.18, 1.44, 1.44 and 0.97 % day-1) and an increase in feed conversion ratio (2.26, 3.11, 4.97, 4.34 and 7.27) respectively. This was largely due to the saponins and other antinutritional factors which affected palatability. Thus there is a need of developing more efficient technique of removing the antinutritional factors in MLM.

Key words: Antinutritional factors, orechromis niloticus, growth, feed utilisation, body composition.

Introduction

Moringa, (Moringa oleifera Lamarck) is a fast growing tree plant which bears long green pods after 6-8 months and production may continue for 30-40 years. It has high forage yield which may amount to 120 metric tonnes dry matter ha^{-1} yr^{-1} (Makkar and Becker, 1999).

Products. from moringa have a wide range of applications in agricultural, industrial and pharmaceutical processes. Moringa leaves have a relatively high crude protein content which varies from 25% (Makkar and Becker, 1996) to 32% (Soliva *et al.,* 2005), A high proportion of this protein is potentially available for digestion due to a high proportion of pepsin soluble nitrogen (82-91 %) and low proportion (1-2%) of acid detergent insoluble protein (Makkar and Becker, 1996). The protein contains high levels of sulphur containing amino acids and compares well with soybean, which is usually regarded as a source of high quality plant protein (Francis *et al.*, 2002). Its crude lipid fraction has a high proportion of n-3 (0)3) fatty acids in the form of linolenic acid which account for almost 67% of total fatty acids (Soliva *et al.,* 2005). The leaves are also rich in vitamins and minerals.

Use of Moringa leaf meal (MLM) as a protein source in fish diets is limited due to presence of high levels of anti-nutritional factors (ANFs), particularly saponins and to a lesser extent tannin, phytic acid and hydrogen cyanide (Francis *et al.*, 200 I). Inclusion of unprocessed MLM in Nile tilapia (Oreochromis niloticus) diets above 12% led to a significant reduction in growth (Richter *et al.*, 2003). However, Makkar and Becker (1996; 1997) showed that significant amounts of anti-nutritional factors, particularly saponins can be removed through solvent and aqueous extractions. Using solvent extracted MLM, the inclusion level could be tripled to 33% without significant effect on fish performance

Accepted July, 2012

(Afuang *et al.,* 2003). Solvent extraction, however, may not be technically and financially feasible for small-scale fish farmers in rural areas. Given the relatively high solubility of saponin (Makkar and Becker, 1996), the current study explored the suitability of aqueous extracted moringa leaf meal as a protein source for Nile tilapia.

Materials and Methods

Processing of moringa leaves

Moringa leaves were harvested during dry season from a moringa tree plot located within the premises of Sokoine University of Agriculture in Tanzania. The leaves were soaked overnight in a tank containing still tap water at 1:1 w/v to remove saponins and other water soluble antinutritional factors. Soaked leaves were placed on a wire mesh to drain excess water and then spread on plastic sheets to dry under shade to avoid loss of vitamins through photodynamic damage/oxidation. The dried leaves were threshed from stalks to reduce crude fibre content in the meal. The dried leaves were then ground into a fine powder using a hammer mill (Lab Mill, screen size 0.2 mm) and stored in plastic bags at room temperature.

Diet formulation

Diets for digestibility trial were formulated as recommended by Cho *et al.* (1982) with Chromium (III) oxide (BDH 277574Q) as an inert marker at an inclusion level of 0.5% as shown in Table 1.

Diets for growth trial were formulated in such a way that processed MLM provided at 0, 15, 30, 45 and 60g $100g^{-1}$ of total dietary crude protein as treatments MLM0, MLM15, MLM30, MLM45 and MLM60, respectively. Diet MLM0 contained fishmeal as a main source of protein and served as control. All diets were formulated to contain 30g 100 g^{-1} crude protein, 18kJ g^{-1} and 10g 100 g^{-1} lipid (Table 2).

Table 1: Formulation of diets used to determine apparent digestibility coefficients of ingredients (g 100g)

Ingredient	Reference Diet	Test diet
Processed moringa 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
Mineral premix [5]	4.00	2.80
Vitamin premix [6]	2.00	1.40
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; $FeSO_4,7H_2O$, 4.00; $ZnSO_4,4H_2O$, 0.88; $MnSO_4,4H_2O$, 0.41; $CuSO_4,5H_2O$, 0.13; CoSO4,$7H_2O$, 0.08; $CaIo_3,6H_2O$, 0.05; CrCl3,$6H_2O$, 0.02 (according to Jauncey and Ross 1982). [6]Contained (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). [7]Carboxymethylcellulose (sodium salt, high viscosity)

Biochemical analysis

Both ingredients and formulated diets were analysed for their biochemical composition according to standard methods (AOAC, 1990). Gross energy was determined using adiabatic bomb calorimeter (Parr, USA) with benzoic acid used as a standard. Amino acid profiles were determined using LKB Biochrom 4151 Alpha plus amino acid analyser (LKB Biochrom Ltd, UK). Phosphorus was quantified using the method outlined by Allen (1989) while other minerals (sodium, potassium, calcium, iron, zinc, manganese, magnesium and copper) were quantified using a Thermo Xseries 2 Inductively Coupled Plasma Mass Spectrophotometer (ICP MS) (Thermo Scientific, USA). Total saponins were determined according to the method described by Baccou *et al.* (1977). Hydrogen cyanide (HCN) was determined as described by Bradbury *et al.* (1999). Tannins were quantified using a procedure described by Allen (1989). Phytic acid was determined using an assay kit (Megazyme, K-Phyt 05/07).

Experimental facilities and fish handling

Digestibility and growth trials were conducted in a recirculation system within the tropical aquarium facility of the Institute of Aquaculture, University of Stirling, Scotland. Nile tilapia fingerlings used were of the red Stirling strain. Fish handling was

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

Ingredient	MLM0	MLM15	MLM30	MLM45	MLM60
Fish meal1	36.0	31.0	26.0	21.0	16.0
Moringa leaf meal	0.0	14.5	29.0	42.0	58.0
Wheat meal2	47.5	38.5	29.5	22.5	11.5
Sunflower oil	6.0	6.0	6.0	6.0	6.0
Mineral premix3	4.0	4.0	4.0	4.0	4.0
Vitamin premix4	2.0	2.0	2.0	2.0	2.0
CMC5	2.0	2.0	2.0	2.0	2.0
α-cellulose	2.0	1.5	1.0	0.0	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

conducted according to United Kingdom Home Office regulations as stipulated by The Animals (Scientific Procedures) Act 1986.

Digestibility trial

Digestibility trial was conducted to determine the apparent digestibility coefficients of processed MLM using indicator method. A total of 4 circular plastic tanks each with 30L capacity were each stocked with 15 Nile tilapia fingerlings with an average weight of 12.89 ± 1.16g. Test and reference diets were fed in duplicates twice a day at 0900 and 1500hrs to apparent appetite. Faecal collection commenced after 7 days to allow evacuation of all previously ingested feed materials. The faeces were collected using modified Guelph system (Cho *et al.,* 1985). One hour after the last meal, the tanks were flushed to remove any uneaten feed and the system was set to collect faeces overnight with water flow reduced to a minimum to facilitate settling of faecal materials.

Next morning all trapped faeces were removed and centrifuged (MSE Centaur 2, Sanyo-Gallenkamp) at 4,300 rpm for 10 minutes. The supernatant was discarded and the faecal material stored in a freezer at -20°C. This procedure was repeated until sufficient faecal material was collected. Before analysis, frozen faecal material was thawed overnight in a refrigerator. Thereafter, the faecal material was dried at 60°C for 48hrs and then ground to a fine powder using mortar and pestle. Chromic oxide was quantified following the method of Furukawa and Tsukahara (1966). Apparent digestibility coefficients (ADC) of MLM were computed as described by Maynard and Loosli (1969) and Lupatsch (2003).

Growth trial

A total of 20 fingerlings with an average weight of 4.68 ± 0.34 g were put into each of 15 self–cleaning circular plastic tanks of 30L capacity. The fingerlings were acclimatized to experimental condition for 1 week during which they were fed trout diet (Nutra Trout Fry 02, Skretting UK). Complete randomised design was used to assign dietary treatments to the tanks and fish were fed to appetite in three equal rations daily at 09:00, 13:00 and 17:00. To ensure minimal wastage, fish were offered the feed in small portions during a period of 10 minutes until they showed no interest in the feed. Fish were bulk weighed once every week for the duration of the trial which lasted for 8 weeks. At the end of 8 weeks final weights were recorded and five fish were randomly sampled from each tank three for whole body proximate analysis and two for histopathology analysis. The remaining fish from each replicate were pooled together according to their dietary treatments for faecal collection to determine digestibility of the diets.

Performance parameters

Performance in growth and nutrient utilisation was 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 protein to digestible energy ratio (DP/DE) as follows:

FI (g fish-1 day-1) = Total feed intake per fish/number of days
ADG (g fish-1 day-1) = final weight – initial weight/number of days
SGR (% day-1) = 100 × (ln[final body weight]- ln[initial body weight])/no. of days
FCR = feed intake/live weight gain
PER = live weight gain/crude protein intake
ANPU (%) = 100 × (final fish body protein (g) - initial fish body protein (g))/crude protein intake (g)
ER (%) = 100 × (final fish body energy - initial fish body energy)/gross energy intake
HSI = 100 × (liver weight/total body weight)
DP/DE ratio (mg DP/kJ DE) = Digestible protein/ digestible energy

Whole body composition was determined through proximate analysis and results expressed as percentage of live weight.

Histopathology of gut and liver

Histological analyses of liver and gut were carried out to examine for any pathological changes due to the dietary treatments. Fish that were sampled for histopathology were dissected to remove the gut and liver which were fixed in 10% neutral buffered formalin. Histological examination of the tissues was performed according to procedures described by Drury and Wallington (1980).

Data analysis

The data was analysed using one way analysis of variance (ANOVA) to determine differences between treatment means which were deemed significant at $P<0.05$. Post-hoc analysis was done where significant differences existed among treatment means using Tukey's Honest Significant Difference Test. Analyses were performed using SPSS software version 13 (SPSS Inc.). Before analysis data were tested for normality using the Kolmogorov–Smirnov test and for homogeneity of variance using Levene's test and transformed accordingly in case of non–conformity. Percentage data was first transformed using arcsine before performing ANOVA.

Results

Biochemical composition and digestibility

The biochemical composition of unprocessed and processed MLM is shown in Table 3. In general, aqueous extraction led to reduction of most nutrients. Crude protein content was reduced by about 10%, though it still remained high (above 30%). Consequently, the amino acid content was also reduced and in particular methionine+cystine was most affected with nearly 67% reduction. The amounts of sodium, iron and zinc were noticeably higher in processed MLM than in unprocessed MLM. On the contrary, less potassium and calcium were observed in processed MLM compared to unprocessed MLM. Aqueous extraction had no effect on saponin content. The contents of other antinutritional factors with the exception of phytic acid were less affected.

Processed MLM was well digested by Nile tilapia as demonstrated by fairly high values of apparent digestibility coefficients and hence the high values of digestible crude protein and gross energy (Table 15.4

The biochemical composition of the diets used for the growth trial is shown in Table 5. A slight variation in proximate composition was observed among the diets. The crude fibre content increased with an increase in MLM with diet MLM60 having almost double the fibre content of diet MLM0. Phosphorus content in diets decreased with increase in MLM inclusion. Levels of antinutritional factors in MLM diets increased with inclusion level of MLM with higher levels observed for phenols, tannins and saponins. MLM diets had high digestibility with apparent digestibility coefficients of above 70%. Generally, apparent digestibility coefficients decreased with increasing levels of MLM in the diets with few exceptions (Table 5).

Levels of digestible protein (DP), digestible energy (DE) and ratios of digestible protein to digestible energy were more or less similar between the MLM diets. However, the intakes of DP and DE (Table 6) significantly declined with increasing inclusion levels of MLM.

Table 3: Biochemical composition of unprocessed and processed MLM

	Unprocessed MLM	Processed MLM
Proximate composition (g 100-1 g, as fed)		
Dry matter	95.8	93.0
Crude protein	34.9	31.1
Crude lipid	5.9	4.5
Crude fibre	7.1	5.9
Ash	8.0	5.5
Nitrogen free extract	39.8	46.1
Gross energy (kJ g-1)	19.9	20.1
Amino acid composition (g 100g-1 feed)		
Arginine	3.2	2.0
Histidine	1.3	0.8
Isoleucine	2.8	1.7
Leucine	4.7	2.9
Lysine	2.3	1.5
Methionine +Cystine	0.8	0.2
Phenylalanine+ Tryptophan	5.0	3.9
Threonine	2.2	1.5
Valine	3.5	2.2
Mineral composition (mg g-1, DM)		
Phosphorus	3.7	3.4
Sodium	0.2	0.4
Magnesium	3.3	3.2
Potassium	15.4	6.2
Calcium	15.0	11.4
Iron	0.1	0.2
Copper	0.0	0.0
Zinc	0.1	0.3
Antinutritional factors (g 100g-1, DM)		
Saponins1	1.2	1.2
Phenols2	4.1	3.4
Tannins2	1.2	1.6
Phytic acid3	0.1	0.2
HCN4	0.2	0.2

1As diosgenin equivalent, 2As tannic acid equivalent, 3As phosphorus equivalent, 4Hydrocyanic acid (mg 100g-1)

Table 4: Apparent digestibility coefficients of processed MLM

	Reference Diet	Test diet	MLM
Proximate composition (g $100g^{-1}$, as fed)			
Dry matter	93.1	94.1	
Crude protein	31.6	32.9	
Crude lipid	10.7	9.7	
Crude fibre	2.8	4.4	
Ash	8.7	8.0	
Nitrogen free extract	39.4	39.2	
Gross energy (kJ g^{-1})	17.9	19.2	
Apparent digestibility coefficients (%)			
Dry matter	73.3	71.8	68.5
Crude protein	84.3	85.9	89.0
Crude lipid	98.4	90.7	65.2
Gross energy	70.1	72.4	76.8
Digestible protein (g $100g^{-1}$)	24.8	26.6	25.7
Digestible energy (kJ g^{-1})	12.6	13.9	15.4

Growth performance and feed utilisation

The difference in body weight between the control diet and the different experimental diets was noticeable after one week while that between diets containing MLM became noticeable after three weeks (Fig. 1).

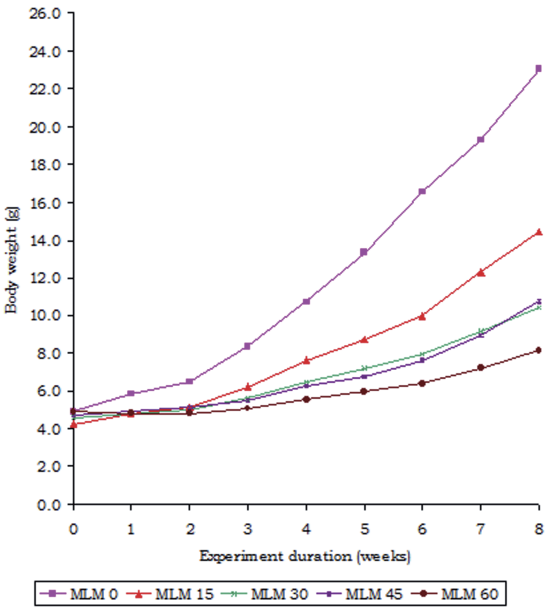

Figure1: Change in body weight of *O. niloticus* fed MLM diets

Inclusion of MLM in experimental diets led to a significant decline in performance of *O. niloticus* as shown in Table 6. Fish showed reluctance to consume diets containing MLM and were even observed to spit out pellets a few times before actual ingestion. This resulted in a significant decline in daily feed intake as well as digestible protein and energy intakes with increasing levels of MLM inclusion. The rejection was more pronounced for diets MLM45 and MLM60. However, it was observed that the fish fed actively on leftovers adhered to the tank sides and exposed to prolonged contact with water. The decline in intake was accompanied by a significant decline in average daily gain (ADG), specific growth rate (SGR) and nutrient utilisation in terms of feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilisation (ANPU), energy retention (ER) and hepatosomatic index (HSI).

Whole body composition

Whole body proximate composition of fish at the beginning and end of the experiment (% fresh weight basis) is shown in Table 7. Final whole body moisture content was significantly higher in fish fed diets with high levels of MLM and the converse was true for the crude lipid and gross energy content. As

Table 5: Biochemical composition and apparent digestibility coefficients of MLM diets used for the growth trial

	MLM0	MLM15	MLM30	MLM45	MLM60
Proximate composition (g $100g^{-1}$, as fed)					
Dry matter	95.2	95.3	95.1	96.5	95.9
Crude protein	32.6	33.7	33.8	34.2	34.2
Crude lipid	10.6	11.0	11.5	12.2	12.3
Crude fibre	3.4	4.0	4.8	4.9	5.8
Ash	9.2	9.2	9.2	9.2	9.1
Nitrogen free extract	39.4	37.5	35.8	36.1	34.5
Gross energy (kJ g^{-1})	19.1	19.4	20.1	20.4	20.6
Phosphorus (mg g^{-1})	7.6	7.4	6.6	6.3	5.7
Amino acids (% Protein)					
Arginine	6.0	6.1	6.2	6.3	6.4
Histidine	2.3	2.4	2.5	2.5	2.5
Isoleucine	4.2	4.4	4.6	4.8	5.0
Leucine	8.0	8.2	8.4	8.5	8.7
Lysine	6.9	6.7	6.4	6.1	5.9
Methionine +Cystine	3.9	3.5	3.0	2.6	2.1
Phenylalanine+ Tryrosine	7.0	7.5	7.9	8.3	8.8
Valine	5.7	6.0	6.2	6.3	6.5
Antinutritional factors (g $100g^{-1}$, DM)					
Saponins1	-	0.2	0.4	0.5	0.7
Phenols2	-	0.5	1.0	1.4	2.0
Tannins2	-	0.2	0.5	0.7	0.9
Phytic acid3	-	0.0	0.0	0.1	0.1
HCN4	-	0.0	0.1	0.1	0.1
Apparent digestibility coefficients (%)					
Dry matter (%)	76.6	78.4	77.3	74.1	78.6
Crude protein (%)	91.0	89.3	85.5	84.6	85.8
Crude lipid (%)	94.3	92.0	88.5	86.5	90.2
Gross energy (%)	79.8	80.2	79.1	77.1	80.6
Phosphorus (%)	75.2	78.0	77.6	74.6	75.1
Digestible protein (DP) ((g $100g^{-1}$)	29.6	30.1	28.9	28.9	29.3
Digestible energy (DE) (kJ g^{-1})	15.2	15.6	15.9	15.8	16.6
DP/DE ratio (mg kJ^{-1})	20.4	20.3	19.1	19.0	18.4

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

a consequence fish fed on diet MLM60 had less than half the body lipid content of those fish fed on diet MLM0. The crude protein content contents varied little between the fish fed the different diets. The ash content, however, declined significantly with increased MLM inclusion.

Histopathology

Examination of liver and small intestine tissues did not show any obvious pathological changes which could be linked to dietary treatment. However, the livers showed a decline in lipid deposition with the increase in inclusion level of dietary moringa leaf meal.

Discussion

The observed reduction of most nutrients after aqueous extraction of moringa leaves was likely due to leaching of soluble nutrients. One of the most noticeable losses was that of crude protein which was reduced by almost 10%. Similar losses in crude protein after aqueous extraction have also been observed in mung bean, Phaseolus aureus (Mubarak, 2005). There is also possibility that the protein loss observed in this study could be attributed to non-protein nitrogen (NPN) similar to that observed in redwood, Acacia villosa, following aqueous extraction (Wina *et al.,* 2005). Moringa leaves are

Table 6: Growth performance and nutrient utilisation of *O. niloticus* fed MLM diets (mean ± SE, n=3)

	MLM0	MLM15	MLM30	MLM45	MLM60
Initial weight (g)	4.92 ± 0.13	4.23 ± 0.15	4.60 ± 0.08	4.72 ± 0.23	4.92 ± 0.06
Final weight (g)	22.86 ± 1.98^{a}	14.29 ± 0.38^{b}	10.31 ± 0.27^{bc}	10.55 ± 0.30^{bc}	8.07 ± 0.27^{c}
Feed intake (g fish-1 day-1)	0.71 ± 0.23^{a}	0.56 ± 0.23^{b}	0.50 ± 0.23^{bc}	0.45 ± 0.01^{cd}	0.40 ± 0.03^{d}
Average daily gain (g fish-1 day-1)	0.32 ± 0.03^{a}	0.18 ± 0.01^{b}	0.10 ± 0.01c	0.10 ± 0.03^{c}	0.06 ± 0.03^{c}
DP intake (g fish-1 day-1)	0.200 ± 0.006^{a}	0.159 ± 0.010^{b}	0.138 ± 0.006^{bc}	0.126 ± 0.001^{cd}	0.114 ± 0.001^{d}
DE intake (kJ fish-1 day-1)	10.28 ± 0.32^{a}	8.22 ± 0.32^{b}	7.62 ± 0.37^{bc}	6.86 ± 0.10^{c}	6.44 ± 0.05^{c}
Specific growth rate (% day-1)	2.73 ± 0.12^{a}	2.18 ± 0.10^{b}	1.44 ± 0.07^{c}	1.44 ± 0.04^{c}	0.97 ± 0.05^{d}
Feed conversion ratio	2.26 ± 0.24d	3.11 ± 0.25^{cd}	4.97 ± 0.37^{b}	4.34 ± 0.15^{bc}	7.27 ± 0.66^{a}
Protein efficiency ratio	1.39 ± 0.13^{a}	0.97 ± 0.08^{b}	0.60 ± 0.04^{cd}	0.68 ± 0.02^{bc}	0.41 ± 0.03^{d}
Apparent net protein utilization (%)	20.85 ± 2.31^{a}	14.82 ± 1.53^{b}	9.10 ± 0.74^{bc}	11.22 ± 0.82^{bc}	6.07 ± 0.51^{c}
Energy retention (%)	12.81 ± 1.83^{a}	9.29 ± 0.77^{b}	4.53 ± 0.43^{c}	5.18 ± 0.09^{c}	2.49 ± 0.06^{d}
Hepatosomatic index	3.01 ± 0.08^{a}	2.32 ± 0.08^{b}	2.15 ± 0.08^{b}	1.62 ± 0.12^{c}	1.54 ± 0.07^{c}
Survival (%).	80.00 ± 13.23	86.67 ± 8.82	86.67 ± 7.26	96.67 ± 3.33	98.33 ± 1.67

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

Table 7: Whole body proximate composition of *O. niloticus* fed MLM diets before and after the experiment (% fresh weight, mean ± SE, n=3)

	Initial	MLM0	MLM15	MLM30	MLM45	MLM60
Moisture content	77.23	73.77 ± 0.89^{a}	74.18 ± 0.45^{a}	76.18 ± 0.45^{ab}	74.54 ± 0.66^{a}	78.35 ± 0.51^{b}
Crude protein	13.39	14.67 ± 0.51	14.70 ± 0.28	14.34 ± 0.28	15.13 ± 0.40	13.98 ± 0.34
Crude lipid	5.60	7.34 ± 0.27^{a}	6.82 ± 0.08^{a}	5.18 ± 0.10^{b}	5.58 ± 0.15^{b}	3.11 ± 0.06^{c}
Ash	3.10	3.07 ± 0.03^{a}	3.24 ± 0.01^{a}	3.65 ± 0.02^{c}	3.77 ± 0.03^{d}	4.09 ± 0.01^{e}
Gross energy (kJ g^{-1})	5.80	5.35 ± 0.01^{a}	5.18 ± 0.01^{a}	4.92 ± 0.02^{b}	4.94 ± 0.01^{b}	4.45 ± 0.08^{c}

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

known to contain appreciable amounts of NPN which ranges between 5% (Makkar and Becker, 1996) and 13% (Makkar and Becker, 1997). However, the loss was contrary to increase protein content following solvent extraction using ethanol (Makkar and Becker, 1996) and methanol (Afuang *et al.,* 2003).

The loss of amino acids could be attributable to the reduction in crude protein content. According to Lyimo *et al.* (1992) loss of protein and other nitrogenous compounds tends to be associated with loss of amino acids. Sulphur amino acids (methionine and cystine) were worst affected with a loss of almost 67% leading to deficiencies in diets MLM30, MLM45 and MLM60 compared to the recommended levels (Santiago and Lovell, 1988). Similarly, the low content of some minerals in the processed MLM could be explained by the reduction in ash due to leaching although they still remained generally high. Generally, the proximate composition of the processed MLM remained within values observed in earlier studies (Makkar and Becker, 1996; Richter *et al.,* 2003; Soliva *et al.,* 2005).

The contents of most anti-nutritional factors remained more or less the same after processing suggesting inefficiency of aqueous extraction used in this study. This is contrary to Makkar and Becker (1997) whose aqueous extraction of moringa leaves reduced saponins and tannins by 93% and 100% respectively. This is likely due to differences on how the process was undertaken. In the current study, moringa leaves were soaked overnight in still tap water at 1:1 w/v while Makkar and Becker (1997) soaked the leaves for 20 minutes in distilled water at 1:50 w/v with stirring. The observed slight increase tannins and phytic acid after processing was similar to that observed in dolichos lablab bean, Lablab purpureus after overnight soaking in water at a ratio of 1:10 w/v (Osman, 2007). According to Vijayakumari *et al.* (1998) the increase in tannin content could be due to degradation of high molecular weight insoluble polymers into smaller molecular weight polymers that give a stronger colour reaction with the reagents. Makkar and Becker (1997) also observed an increase in phytic acid content after aqueous extraction of moringa leaves but contents in the current study remained very low in any case. The level of HCN in both raw and processed MLM (1.58 mg kg^{-1}) was below 'safe' level of 100 mg HCN kg^{-1} recommended by the European Union Commission.

The crude protein ADC of 89% was within limits regarded as high (75%-95%) by Cho and Kaushik (1990) indicating the ability of Nile tilapia to digest the moringa leaf protein. The ADC for crude lipid (65%) was, however, low compared to the routinely reported range of 85–95% (NRC, 1993). This is likely to be due significant amounts of indigestible waxes which are contained in the lipid fraction of MLM (Afuang *et al.,* 2003). The fairly high digestibility of moringa leaf was reflected in the values of digestible protein and energy which were high and comparable to the values reported by Anderson *et al.* (1991). The good digestibility of moringa leaf meal could explain the high digestibility coefficients for the MLM diets used in the growth trial. Consequently DP and DE values were high with the DP/DE ratio above the optimal value of 18 mg kJ^{-1} recommended for Nile tilapia (Kaushik *et al.,* 1995).

The observed reduced feed intake was perhaps one of the most important factors responsible for poor growth of fish used in this study. The presence of antinutritional factors, particularly saponins and tannins, could be responsible for lowering palatability due to their astringent/bitter taste (Francis *et al.,* 2001; Makkar, 2003). Similarly, poor feed intake of diets containing saponin and/or tannins in Nile tilapia has been observed by Afuang *et al.* (2003) and Dongmeza *et al.* (2006).

However, it is likely that saponins played a bigger role than tannins in reducing palatability. When Al–Owafeir (1999) fed diets containing graded levels of saponins (0.08-0.42 $100g^{-1}$) and tannins (0.05-0.71g $100g^{-1}$) to Nile tilapia, significant reduction in feed intake was only observed in diets containing saponin. This supports the observation of active feeding on feed from the previous meal that had adhered to the tank sides where additional leaching of soluble antinutritional factors, like saponins, could have improved palatability. Drying and/or grinding of moringa leaves before aqueous extraction might improve the removal of soluble antinutritional factors.

Another reason for the poor performancecould be the deficiency of sulphur amino acids in some diets. Methionine is essential for normal growth while cystine is a conditionally indispensable amino acid which has a sparing effect on methionine (Wilson, 2002). This deficiency could have been further complicated by the fact that availability of sulphur amino acids in diets containing plant ingredients tend to be lower than for most of the other amino acids (Swick, 1995).

The observed higher body moisture content of fish fed high levels MLM is in agreement to the results reported for carp fed diets containing high amounts of plant proteins such as mustard oilcake, linseed and sesame meal (Hossain, 1988). The decreased body lipid content was probably due to poor feed intake which resulted in starvation and in turn led to mobilization of body lipid reserves to meet energy requirements for vital body functions. The presence of saponins may also have contributed to inhibited pancreatic lipase activity and hence delayed intestinal absorption of dietary fat (Han *et al.,* 2000). The increase in ash with increased MLM could also be linked to starvation.

Conclusion

Overnight aqueous extraction of moringa leaves using tap water is not effective in removing saponins and other soluble antinutritional factors led to the observed poor diet palatability and a significant reduction in feed intake. However, high digestibility and lack of any obvious histopathological signs of abnormality in liver and intestine suggest that MLM has potential to serve as a protein source for O. niloticus. This, however, will depend on the efficiency of removing antinutritional factors to improve palatability and thus increase feed intake. Further studies are needed to explore more potent means of removing the antinutritional factors.

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