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Replacing Fishmeal with Kikuyu Grass and Moringa Leaves: Effects on Growth, Protein Digestibility, Histological and Haematological Parameters in *Clarias gariepinus*

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Abstract

The effects of replacing fishmeal (FM) with kikuyu meal (KM) and moringa meal (MM) in *Clarias gariepinus* diets were investigated. Nine diets (30% CP: 20 MJ kg^{-1}) were formulated by replacing FM with KM and MM at 0% (control), 25, 50, 75 and 100%. Each diet was randomly assigned to triplicate groups of *C. gariepinus* (30.5±2 g) for 60 days. Significantly higher ($P<0.05$) growth performance, feed and protein utilisation was observed in *C. gariepinus* fed KM diets compared to those fed MM. No histological alterations were found in liver of fish fed the control diet. Increased hepatocyte degradation was seen in fish fed higher levels of moringa in the diet. The enterocytes showed a significant increase in the number of goblet cells with increasing levels of MM. Villi height decreased significantly ($P<0.05$) when MM replaced >75 fishmeal. Higher levels of anti-nutrients in MM may be the cause of the adverse effects in growth, histology and haematological parameters of fish fed moringa based diets. The results of this study indicate that KM can replace up to 25% FM and that adding MM resulted in reduced performance. Higher profit index and lower incidence cost was observed KM diets than in MM diets.

Keywords: Feed utilisation, blood biochemistry, intestine, liver.

Introduction

The availability of fishmeal (FM) which is the main protein source for fish feeds can no longer be guaranteed because the capture fisheries are levelling off (FAO, 2011). As a result, the price of FM is constantly rising, adversely affecting the profitability of aquaculture enterprises (Sintayehu *et al*., 1996). This has forced the aquaculture industry to explore alternative, cheaper protein sources for use as FM replacers in aquafeeds. The decrease in global production of FM clearly demonstrates that the sustainability of this industry will depend on the sustained supply of plant proteins for aquafeeds. Soybean meal has been the main plant protein source used in animal feeds as a replacement for FM because of its high protein content and relatively well balanced amino acid profile (Sintayehu *et al*., 1996). However, soybean meal has been increasingly commercialised and its use as the main protein source in fish feeds may longer be economically viable.

In this study, the effect of replacing dietary fishmeal with kikuyu grass (*Pennisetum clandestinum*) and moringa (*Moringa oleifera*) on the performance of *Clarias gariepinus* was determined. The leaves of both plants have moderately high protein content and amino acid profiles. Kikuyu grass is a common forage, used widely as a lawn grass (Marais *et al*., 1987). Originating from former Zaire (now Democratic Republic of Congo) and Kenya, this grass has been introduced widely in tropical areas, including Costa Rica, Hawaii, Colombia, Australia and southern Africa. There is scarcity of information on the use of kikuyu grass in fish feeds. Work done in *Tilapia rendalli* indicate that this grass can replace up to 25% FM (Hlophe and Moyo, 2014). *Moringa oleifera* Lam., is a member of the family Moringaceae. It is a fast-growing plant widely available in the tropics and subtropics with economic importance for the food and medical industry. An appreciable amount of work has been done on the use of moringa leaf meal as a protein source in tilapia diets (Afuang *et al*., 2003; Richter *et al*., 2003). However, for a plant protein source to be included in aquafeeds its utilisation should be tested in different fish species because fish species differ in their sensitivity and response to anti-nutrients found in plant protein sources (Francis *et al*., 2001; Alarco´n *et al*., 2002; Chong *et al*., 2002; Gatlin *et al*., 2007; Collin *et al*., 2012; Chaudhuri *et al*., 2012).

Clariidae catfishes are the second most important group of cultured fish in the world

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(Fasakin *et al*., 2003). They feed on a wide range of artificial and natural food items, have high growth rates and tolerate poor water quality parameters (Amisah *et al*., 2009). High activities of protease, lipase and amylase enzymes in *C. gariepinus* digestive tract indicate its ability to utilise both animal and plant based feed resources (Hlophe *et al*., 2014). Fishmeal free formulations for herbivorous fish such as tilapias have been reported and used in practical diets (Shiau *et al*. 1990), but seldom studied on other omnivorous fish. Previous studies on replacing FM with plant protein sources in *C. gariepinus* diets focussed mainly on growth performance and feed utilisation (Bichi and Ahmad, 2010; Amisah *et al*., 2009). The intestines and liver are key organs in digestion and absorption of nutrients from food, therefore, the monitoring of these organs is imperative in nutrition studies (Raskovic *et al*., 2011). Thus, the aims of this study were to evaluate growth performance, protein utilisation and digestibility in *C. gariepinus* fed kikuyu and moringa based diets. Additionally, the effects of these diets on liver and intestine histology as well as blood parameters were determined.

Materials and Methods

Feed Preparation

Kikuyu grass was harvested from the grounds (lawn) at the Aquaculture Research Unit, University of Limpopo, South Africa. Moringa leaves were sourced from the Patient Wellness Centre in Lebowakgomo in the Limpopo Province of South Africa. The kikuyu grass and moringa leaves were dried under shade and milled using a hammer mill. The proximate composition of the FM and dried leaves; kikuyu meal (KM), moringa meal (MM) used is given in Table 1. Nine isonitrogenous (crude protein 30% DM) and isocaloric (gross energy 20 MJ/kg DM) diets were formulated by replacing FM with KM and MM. The control diet contained no plant meal and 10.62% FM. In the experimental diets FM was substituted at 25, 50, 75 and 100% with KM in diets KM 25, KM 50, KM 75 and KM 100 respectively. In diets designated as MM 25, MM 50, MM 75 and MM 100 fishmeal was replaced with MM at 25, 50, 75 and 100%. The level of maize and maize gluten was adjusted accordingly. In each diet, chromic oxide (Cr_2O_3) was added at 0.5% as an inert marker. The diets were formulated using Winfeed 3, EFG (Natal) program.

Experimental Procedures

A completely randomised design experiment was set up with twenty seven rectangular (1.5 m^3) fibre glass tanks housed in a greenhouse for each species. Each tank was filled with aged water to the 1 m^3 mark. All tanks were connected to a recirculating system. An Elektror side channel blower Model: D7300 (Karl W. Miller) was used to blow air which was diffused with an air stone into each tank. Each diet was randomly assigned to triplicate groups of *C. gariepinus* (30.50±2 g), stocked at 20 fish per tank. All fish were fed their allotted diet three times a day (0900, 1300 and 1700 hours) for 60 days to apparent satiation (one pellet remains uneaten for 1–2 minutes) the amount of feed consumed in each tank was recorded. Faecal samples were siphoned from

Table 1. Proximate, amino acid and anti-nutrient composition of kikuyu, moringa leaf meals, fishmeal used and amino acid requirements for *Clarias gariepinus*

Components	KM	MM	FM	Amino acid requirements for channel catfish*
Dry matter (%)	93.63	92.00	90.50	
Protein (%)	26.84	32.09	64.80	
Energy (MJ kg^{-1})	17.99	18.30	12.90	
Ash (%)	9.50	7.17	21.10	
Fat (%)	4.38	1.99	5.60	
Fiber (%)	18.58	10.10	0.00	
Methionine + cysteine (%)	0.88	0.58	3.86	0.64
Arginine (%)	1.44	1.20	5.82	1.2
Histidine (%)	0.51	0.58	2.43	0.42
Threonine (%)	0.93	0.80	4.31	0.56
Isoleucine (%)	1.12	0.87	4.68	0.73
Leucine (%)	2.33	2.05	7.60	0.98
Phenylalanine + tyrosine (%)	2.14	2.38	7.61	1.40
Lysine (%)	1.42	1.10	7.57	1.43
Valine (%)	1.56	1.10	5.25	0.84
Anti-nutritional factors (g/kg)				
Polyphenols	14.82	43.02	-	
Tannins	8.40	12.10	-	
Phytate	6.42	25.41	-	
Saponins	20.31	79.32	-	

*Amino acid requirements of a related fish species – channel catfish (NRC, 1993)

each tank 2 hours after each feeding and stored in a freezer (-20°C) until there was enough sample for analysis. Water temperature ranged between 25.6 and 28.4°C, dissolved oxygen ranged between 6.00 and 6.80 mg L^{-1}, pH 7-8.2 and the photoperiod was natural.

Proximate Composition of Experimental Diets and Faeces

Proximate composition of the FM, KM, MM (Table 1) and all the experimental diets (Table 2) was analysed following the Association of Official Analytical Chemists procedures (AOAC International, 2012). All samples were analysed for dry matter, crude protein, crude lipid, crude fibre, gross energy and ash. Each sample was dried for 24 hours at 105°C for dry matter determination. Nitrogen content of the dry matter was determined using a LECO FP2000 Nitrogen Analyser which uses the Dumas combustion. The protein content was calculated as % nitrogen x 6.25. The lipid content was measured by Soxhlet extraction of freeze-dried samples with petroleum ether at 50°C. Gross energy was determined with the aid of a DDS isothermal CP 500 bomb calorimeter. Crude fibre was determined as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH. The polyphenols and tannins in KM and MM were determined using the spectrophotometric methods as described by Makkar *et al*., 1993). Quantitative determination of phytates and saponins was done using the method of AOAC (2012). Amino acids determination was done on a Beckman Amino Acid Analyser System 6300. All tests were done in triplicate.

Growth Performance, Feed Utilisation Indices and Protein digestibility

Growth rate defined as thermal-unit growth coefficient (TGC), was calculated as: TGC = 100 x ($FBW^{-0.333}$-$IBW^{-0.333}$)/(∑T°Cxdays), where FBW = final body weight (g/fish); IBW= initial body weight (g/fish); T = temperature (Iwama and Tautz, 1981). Specific growth rate (SGR) was calculated according to Winberg (1956) as: SGR = (InW_f-InW_0)/t, where: W_f = final body weight (g), W_0 = initial body weight (g), In = natural Logarithm $(log)^{-10}$, t = feeding period (days). Feed utilisation was determined using feed conversion ratio (FCR) = [food consumed (g)]/ [weight gained (g)] and protein efficiency ratio (PER) = [weight gained (g)]/[protein intake (g)].

The apparent digestibility coefficient (ADC; %) of protein was determined using the indirect method which uses Cr_2O_3 as an inert marker (Cho *et al*., 1982). ADC = 100 [1-(Cr_2O_3 in diet)/Cr_2O_3 in faeces) x (protein in faeces/protein in diet)].

Histological Analysis, Haematological Analysis and Cost Benefit Analysis

At the end of the experimental period, five fish from each tank were sacrificed for histological analysis of intestines and the liver. Liver samples and 1 cm segments from the mid gut of the intestinal tract were preserved in 10% neutral buffered formalin for 24 hours. Subsequently, liver and intestine tissues were dehydrated using standard histological techniques in graded ethanol series and embedded in paraffin wax for histology. From each sample, 3-5 µm sections were cut and mounted on glass slides before

Table 2. Ingredients (g/kg) and proximate composition of experimental diets

	Control	KM25	KM50	KM75	KM100	MM25	MM50	MM75	MM100
FM (%)	0	25	50	75	100	25	50	75	100
KM	_	7.50	15.00	22.50	30.00	_	_	_	_
MM	_	_	_	_	_	7.27	14.72	21.70	29.00
Fish meal	10.62	7.73	5.25	2.77	_	8.00	5.31	2.70	0.00
Soybean	7.32	7.32	7.32	7.32	7.32	7.00	7.00	7.00	7.00
Canola	18.00	18.00	18.00	18.00	18.00	18.71	18.71	18.71	18.71
Sunflower	16.80	16.80	16.80	16.80	16.80	16.80	16.80	16.80	16.80
Maize gluten	11.60	12.00	12.00	12.00	12.00	11.60	11.60	11.60	11.60
Wheat middling	2.17	2.17	2.17	2.17	2.17	5.00	5.00	5.00	5.00
Maize	28.56	23.45	18.37	13.30	8.25	18.08	13.25	8.73	4.39
Canola oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Binder	2.00	2.00	2.00	2.00	2.00	4.00	4.00	4.00	4.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Crude protein (% DM)	30.03	29.87	29.96	29.71	30.07	29.84	29.98	30.48	30.38
Gross energy (MJ/kg DM)	20.21	20.34	20.48	20.53	20.47	20.58	20.42	20.26	20.36
Fat (% DM)	3.94	4.65	4.52	4.31	3.93	3.60	3.78	3.84	3.35
Crude fibre (% DM)	7.04	8.07	8.88	10.31	12.91	7.03	7.59	7.88	8.54
Dry matter (%)	94.69	92.40	92.32	91.32	91.40	94.65	95.22	95.47	94.84

FM- fishmeal; KM- kikuyu meal; MM- moringa meal

[1] Mineral premix (g kg^{-1}): KH_2PO4, 502; $MgSO_4$. $7H_2O$, 162; NaCl, 49.8; $CaCO_3$, 336; Fe (II) gluconate, 10.9; $MnSO_4$. H_2O, 3.12; $ZnSO_4$. $7H_2O$, 4.67; $CuSO_4$. $5H_2O$, 0.62; KI, 0.16; $CoCl_2$. $6H_2O$, 0.08; ammonium molybdate, 0.06; $NaSeO_3$, 0.02.

[2] Vitamin premix (g or IU kg^{-1} premix); thiamine, 5; riboflavin, 5; niacin, 25; folic acid, retinol palmitate, 500,000 IU;1; pyridoxine, 5; cyanocobalamine, 5; cholecalciferol; 50,000 IU; a-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; ascorbic acid, 10; choline chloride, 100; biotin, 0.25.

staining with haematoxylin and eosin. Slides were examined under light trinocular microscopy at 400X (Leica Microsystems model DM750, Leica, Bannockburn, IL, USA). Each slide was photographed with a DVC digital camera (Digital Video Camera Company, Austin, TX) mounted on a BH-2. Twenty measurements for villi height and width (µm) were taken from each intestine slide using Image J (1.46) software. Baeverfjord and Krogadahl (1996)'s method was used to count goblet cells in each segment. Liver degradation was quantified by examining the hepatocyte nuclei, vacuolisation and cytoplasm according to McFaden *et al*. (1997). Each liver specimen was assigned one of 3 grades (1-3), a healthy specimen scoring 1 and a degraded liver scoring 3 (Table 3). The mean score of all samples in that treatment was expressed as the hepatocyte degradation value.

Blood was drawn through caudal venous puncture from five fish in each tank (15 fish per treatment) for haematological analysis. The analysis for white blood cells (WBC), red blood cells (RBC), haematocrit (HTC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) was done on the Systemex, XT-1800i blood analyser. Plasma glucose, plasma protein and blood urea nitrogen (BUN) were analyzed on a 600 DxC General Chemistry Analyser.

The following economic indicators were calculated based on the assumption that all operating costs remained constant and that only the cost of ingredients was variable (Bahnasawy *et al*., 2003): Incidence cost = cost of feed / quantity of fish produced (kg); Profit index = local market value of fish / cost of feed.

Statistical Analysis

Normality and homogeneity of variance was tested using the Shapiro-Wilk normality test. Linear regression equations of SGR, TGC, FCR, PER, protein digestibility on leaf meal inclusion rate were calculated. Analysis of covariance (ANCOVA) was used to test if the regressions between the plant diets were significant for each parameter. One way analysis of variance (ANOVA) was used to determine the effect of diet on histology and haematology parameters. Significance was accepted at probabilities ≤0.05. All statistical analyses were conducted using SAS for windows (SAS Institute, 9.2: 2008).

Results

Growth Performance Indices and Anti-Nutrients in Leaf Meals

All feeds were accepted at the start of the feeding trial, however, feed intake was significantly ($P<0.05$) reduced as the levels of both leaf meals in the diet increased. The highest TGC and SGR were recorded in *C. gariepinus* fed the control diet. Growth performance indices; TGC and SGR decreased linearly with increasing levels of both KM and MM in the diet (Table 4a and b). Fish fed KM based diets had significantly higher ($P<0.05$, ANCOVA) feed intake, TGC and SGR than those fed the MM diets.

The best FCR was observed in fish fed the control diet and increased linearly with inclusion of both leaf meals and this increase was significant ($P<0.05$). The FCR of fish fed KM diets was significantly ($P<0.05$, ANCOVA) better than that of fish fed MM diets (Table 4a and b). Protein utilisation was significantly reduced ($P<0.05$) when levels of both leaf meals increased in the diet (Table 4a and b). The PER of *C. gariepinus* fed KM based diets was significantly higher ($P<0.05$) than that of fish fed MM based diets. Apparent digestibility coefficient for protein also decreased as the level of both KM and MM in the diet increased and was higher in fish fed KM than those fed MM diets (Table 4 a and b). The Saponin concentrations were higher in MM (79.32 g kg^{-1}) than in KM (20.31 g kg^{-1}). Polyphenols, tannins and phytate concentrations were also higher in MM than in KM (Table 1).

Effect of Kikuyu and Moringa Diets on the Liver Condition and Intestine Histology

Histological analysis of *C. gariepinus* fed the control diet showed hepatocytes of regular shape with large centrally located nuclei and homogenous cytoplasmic lipid content showing distinct boundaries (Figure 1). A slight degradation in hepatocytes

Table 3. Histological criteria used for scoring of *C. gariepinus* hepatocytes

Tissue	Grade		
	1 (Healthy)	2 (Intermediate)	3 (Degraded)
Liver nuclei	Lightly granular, small and distinct	Abundant granules, enlarged or indistinct nucleoli	Small dark and pyknotic
Cytoplasm	Structured: Varied texture, scattered granules with eosin positive patches	Homogenous: Granular slight variability in staining property	Hyaline: Lacking texture, dark small and often separated the cell boundary
Fatty deposits	Large fatty deposits which follow cell boundary and encroach on the nucleus	Small occasional fatty deposits	No fatty deposits, cells shrink leading to large sinusoidal space.

Adapted from McFadzen *et al* (1997).

Table 4. Effect of (a) kikuyu and (b) moringa leaf meals on *Clarias gariepinus* growth performance, protein digestibility and cost benefit analysis

(a)

	Control	KM 25	KM 50	KM 75	KM 100	*P* model	r^2
IBW (g)	30.51±3.10	31.65 ± 1.65	31.27±2.12	30.45 ± 0.55	31.40 ± 0.80		
FBW (g)	150.25±1.5	142.14±2.2	120.31±3.6	106.22±2.5	96 .50±1.1		
Feed intake (g/fish/day)	1.58±0.2 ^{a}	1.48±0.6 ^{b}	1.34±0.1 ^{c}	1.28±0.2 ^{d}	1.23±0.5^{e}	0.003	0.951
TGC	0.13±0.1^{a}	0.12±0.1^{a}	0.11±0.1^{ab}	0.10±0.1^{b}	0.08±0.1^{c}	0.004	0.987
SGR (%/day)	2.8±0.3 ^{a}	2.5±0.5 ^{ab}	2.25±0.6^{b}	2.08±0.1^{c}	1.85±0.7^{d}	0.001	0.988
FCR	0.8±0.1 ^{a}	0.82±0.3^{a}	0.90±0.2^{b}	0.99±0.4^{c}	1.14±0.1^{d}	0.008	0.908
PER	4.19±0.5^{a}	4.12±0.9^{a}	3.70±0.10.5^{b}	3.36±0.2^{c}	2.93±0.4^{d}	0.003	0.951
ADC (Protein)	83.21±1.1^{a}	82.24±0.5^{ab}	80.07±1.2^{b}	79.69±1.4^{bc}	77.84±0.9^{c}	0.001	0.979
Incidence cost	0.01	0.01	0.01	0.01	0.01		
Profit index	2.02	2.07	2.18	2.26	2.36		

(b)

	Control	MM 25	MM 50	MM 75	MM 100	*P* model	r^2
IBW (g)	30.51±3.10	30.25±1.5	31.01±1.8	30.59±0.9	29.56±1.1		
FBW (g)	150.25±1.5	123.17±1.3	111.00±3.5	106.21±2.5	86.03±1.2		
Feed intake (g/fish/day)	1.58±0.2^{a}	1.30±0.3^{b}	1.24±0.7^{c}	1.25±0.3^{c}	1.14±0.4^{f}	0.045	0.786
TGC	0.13±0.1^{a}	0.11±0.1^{ab}	0.10±0.1^{b}	0.09±0.1^{c}	0.08±0.1^{d}	0.002	0.964
SGR (%/day)	2.8±0.3^{a}	2.3±4.02^{b}	2.01±0.3^{c}	1.8±0.2^{d}	1.58±0.4^{e}	0.002	0.959
FCR	0.8±0.1^{a}	0.84±0.2^{a}	0.94±0.1 ^{b}	1.03±0.2 ^{c}	1.21±0.5 ^{d}	0.005	0.929
PER	4.19±0.5^{a}	3.96±0.6^{b}	3.58±0.5 ^{b}	3.55±0.2 ^{c}	2.76±0.2 ^{e}	0.013	0.902
ADC (Protein)	83.21±1.1 ^{a}	81.53±1.3^{b}	78.81±0.7^{c}	76.62±0.5^{d}	74.50±1.2e	0.001	0.875
Cost benefit analysis							
Incidence cost	0.01	0.01	0.01	0.02	0.02		
Profit index	2.02	1.79	1.66	1.55	1.44		

Values are mean of triplicate determinations ± standard deviations. Values in the same rows with the same superscripts are not significantly different (P>0.05).

integrity was observed with increasing levels of KM in the diet. The hepatocytes of fish fed high levels of MM in the diet had poorly visible cell membranes and hyaline cytoplasm, small pyknotic nuclei pushed to the periphery of the cell and higher incidence of Kupffer cells (Figure 1). Fish fed up to 75% KM did not show any significant increase in hepatocyte degradation scores (Figure 2). Fish fed MM had higher degradation scores than those fed KM at the same replacement level (Figure 2). Hepatocytes of *C. gariepinus* fed MM based diets were unevenly shaped with smaller nuclei and showed significantly higher (P<0.05) hepatocyte degradation when MM replaced >25% of FM. *C. gariepinus* fed MM100 had the highest hepatocyte degradation score of 3.00 (Figure 2). Villi height decreased with increasing levels of KM and MM in the diet (Table 5). In KM fed fish, this decrease was only significant (P<0.05) at the highest inclusion level (KM 100). In the fish fed MM based diets, this decrease was significant (P<0.05) in fish fed diets MM 75 and MM 100. Furthermore, a significant increase in goblet cells number was observed in treatment groups (Figure 3). Goblet cell number was significantly higher (P<0.05) when MM replaced more than 50% of FM in *C. gariepinus* diets (Table 5).

Effect of Diet Kikuyu and Moringa Diets on Haematological Parameters

WBC in *C. gariepinus* increased with increasing plant meal in the diet. This increase, though not significant (P>0.05) was more pronounced in MM diets (Table 6). *C. gariepinus* RBCs and HCT were not significantly affected at all levels of KM inclusion, but were significantly lower in in fish fed MM100. Mean corpuscular volume, MCH and MCHC were not affected by dietary treatment. No significant (P>0.05) trend was observed in plasma glucose of *C. gariepinus* fed the experimental diets. Plasma protein and BUN decreased with increasing KM level, this was not significantly lower (P>0.05) than the control in all KM diets. In the MM diets, plasma protein and BUN also decreased at higher MM levels and were significantly lower (P<0.05) than the control in fish fed MM50 - MM100 (Table 6).

Cost Benefit Analysis

The cost benefit analysis of this experiment show that adding KLM in *C. gariepinus* diets resulted in lower incidence cost and higher profit index when compared to the control (Table 4). The inclusion of MLM on the other hand, resulted in increasingly higher incidence costs and lower profit index with increasing levels of MM in the diet (Table 4).

Discussion

The growth performance parameters (TGC and SGR) in *C. gariepinus* fed the experimental diets decreased as the level of both leaf meals in the diet increased. The decrease in growth performance corresponds to a decrease in feed intake. FM has been traditionally used as the main protein source in fish diets because of its high protein content, well balanced amino acid profile and high palatability (FAO, 2011; Hlophe and Moyo, 2011). Therefore, as

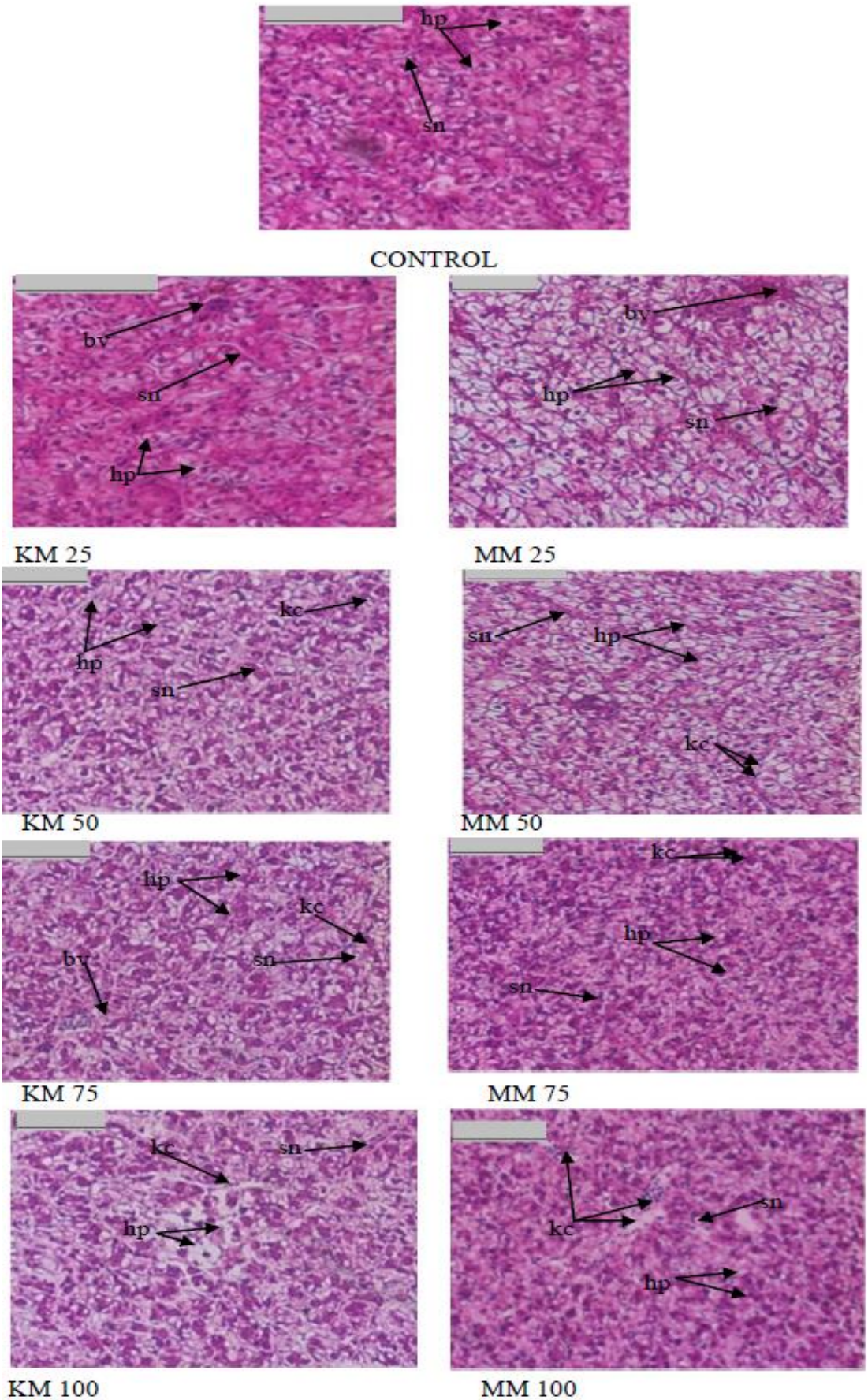

Figure 1. Effect of kikuyu and moringa leaf meals on liver histology in *Clarias gariepinus*. Scale bar = 100 µm. arrows point to hepatocytes (hp), sinusoids (sn), Kupffer cells (kc) and blood vessels (bv).

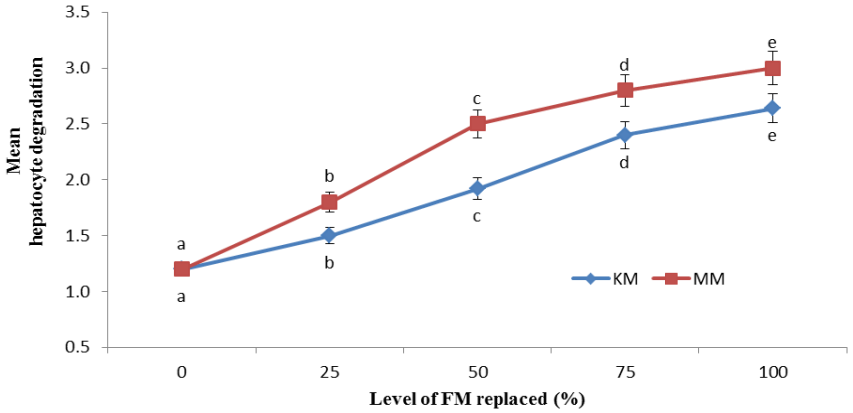

Figure 2. Mean hepatocyte degradation of *Clarias gariepinus* fed different inclusion levels of kikuyu and moringa leaf meals. Data points on a curve with different letters are significantly different (P=0.05). Bars represent standard deviation.

Table 5. Intestine histology values of *Clarias gariepinus* fed different inclusion levels of kikuyu and moringa leaf meals

	% of fishmeal replaced by the plant meals in the diet				
	Control	25	50	75	100
Villi length (µm)					
KM	634.48±10^{a}	624.51±6^{a}	617.41±8^{a}	602.55±9^{a}	600.38±12^{b}
MM		621.75±10^{a}	614.52±10^{a}	593.89±30^{b}	576.75±13^{b}
Villi width (µm)					
KM	83.12±10^{a}	82.71±14^{a}	82.89±12^{a}	83.74±9^{a}	83.38±12^{a}
MM		84.02±11^{a}	83.59±13^{a}	82.50±11^{a}	83.45±15^{a}
Goblet cell number					
KM	450.02±14^{a}	462±15^{a}	478±12^{a}	492±10^{b}	503±14^{b}
MM		485±10^{a}	498±7^{b}	502±11^{b}	527±16^{b}

Values are mean of five determinations ± standard deviations. Values in the same rows with the same superscripts are not significantly different (P>0.05).

Table 6. The Effect of the experimental diets on haematological parameters of *Clarias gariepinus*

	CNTL	KM 25	KM 50	KM 100	MM 25	MM 50	MM 100
WBC (10^3 µl)	609.80±9.2^{a}	615.81±9.6^{a}	617.64±7.1^{a}	625.24±8.7^{b}	611.87±9.8^{a}	629.74±8.5^{b}	635.21±7.2^{b}
RBC(10^6 µl)	2.85±0.19^{a}	2.72±0.17^{a}	2.67±0.2^{a}	2.68±0.15^{a}	2.65±0.20^{a}	2.64±0.19^{a}	2.62±0.15^{b}
HTC (l/l)	0.39±0.1^{a}	0.37±0.1^{a}	0.35±0.2^{a}	0.32±0.1^{a}	0.36±0.1^{a}	0.34±0.2^{a}	0.31±0.1^{b}
Hb (g/dl)	10.33±2.1^{a}	10.32±1.5^{a}	10.30±2.7^{a}	10.28±1.9^{a}	10.25±2.2^{a}	10.25±2.1^{a}	10.26±2.8^{b}
MCV (fL)	136.84±7.5^{a}	136.03±5.6^{a}	131.10±4.5^{a}	119.40±7.1^{a}	135.85±9.6^{a}	128.79±4.8^{a}	118.32±7.2^{a}
MCH (pg)	36.24±2.1^{a}	37.94±3.1^{a}	38.60±2.1^{a}	38.36±2.5^{a}	38.83±2.8^{a}	38.83±1.5^{a}	39.10±3.8^{a}
MCHC (g/dl)	26.48±2.4^{a}	27.89±3.5^{a}	29.40±1.6^{a}	32.13±2.3^{a}	28.47±4.1^{a}	30.14±2.5^{a}	33.09±3.5^{a}
Glucose (g/dl)	2.40±0.2^{a}	3.40±1.2^{a}	2.70±0.1^{a}	1.80±0.3^{a}	1.80±0.2^{a}	3.00±0.2^{a}	2.40±0.3^{a}
Total protein (g/l)	41.00±1.1^{a}	40.00±2.3^{a}	39.60±2.1^{a}	34.00±1.5^{b}	39.00±1.0^{a}	35.00±2.0^{b}	34.50±1.4^{b}
BUN	1.00±0.1^{a}	0.95±.02^{a}	0.80± 0.1^{a}	0.85± 0.1^{a}	0.85± .011^{a}	0.75±0.2^{b}	0.70±0.1^{b}

Values are mean of five determinations ± standard deviations. Values in the same rows with the same superscripts are not significantly different (P>0.05).

WBC- white blood cells; RBC- red blood cells; HCT- hematocrit; Hb- hemoglobin; MCV- mean corpuscular volume; MCH- Mean corpuscular hemoglobin; MCHC- mean corpuscular haemoglobin concentration; Glucose- plasma glucose; BUN- Blood urea nitrogen.

the amount of FM in the diet decreased, the palatability may have also decreased hence the lower feed intake observed. Furthermore, plant protein sources contain anti-nutrients which reduce palatability (Francis *et al*., 2001). This may result in an interaction between the pro-nutritional effects of FM and the anti-nutritional effects in the leaf meals. The higher levels of anti-nutrients in moringa than in kikuyu grass may explain the lower palatability and poor growth of fish fed the moringa based diets. Similar results have been reported by Bairagi *et al*. (2004) who used leaf meals to replace fishmeal.

Protein efficiency and digestibility was highest in fish fed the control diet. When FM was replaced with KM and MM, protein efficiency and digestibility decreased. Higher PER and protein ADC in KM based diets compared to MM diets indicate that KM is digested and utilised better than MM. Apparent digestibility coefficient for *C. gariepinus* fed up to 50% KM fell within limits regarded as high (75-95%). These high digestibility values are in the same range with values reported in *C. gariepinus* fed soybean

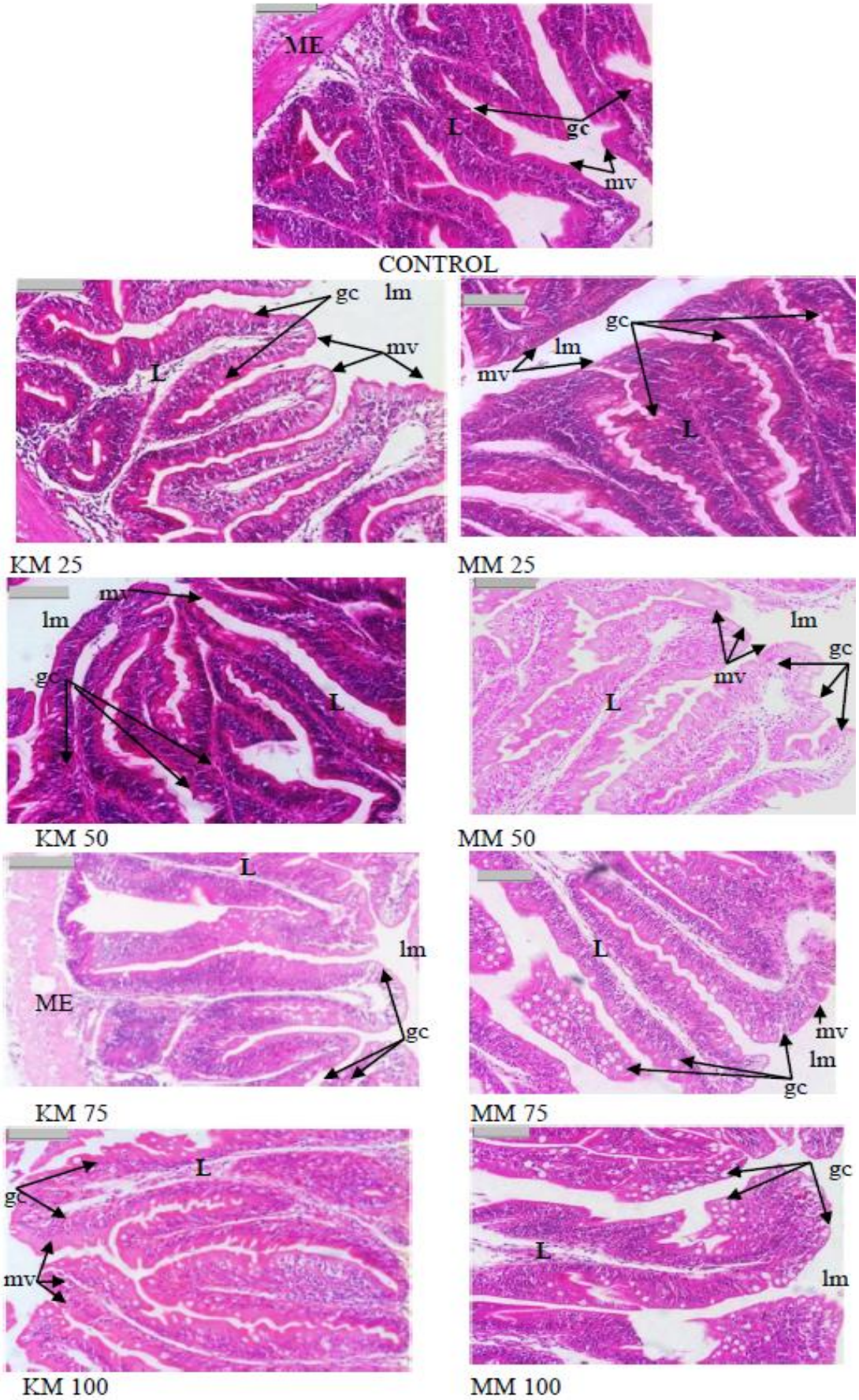

Figure 3. Effect of kikuyu and moringa leaf meals on intestine histology of *Clarias gariepinus*. Scale bar = 100 µm. ME - muscularis externa, L - lamina propria, lm - lumen; arrows point to microvilli (mv), goblet cells (gc).

based diets (Fagbenro and Davies, 2001). However, protein ADC values in MM based diets were below this range. These results suggest that KM may be a viable protein source in *C. gariepinus* diets as it is widely available and not as highly priced as soybean.

The amino acid (AA) profile is an important factor considered in selecting a protein source in fish diets (El-Sayed, 2006). In both plant meals used in this study, the AA profile was sufficient to meet *C. gariepinus* dietary requirements. The poor performance (growth, protein utilisation and digestibility) of fish fed MM diets may be attributed to the negative effects of anti-nutrients. According to Eusebio *et al*. (2004) the presence of anti-nutrients may hinder the digestibility and utilisation of dietary nutrients. Phytate reduces the bioavailability of nutrients due to formation of complexes such as a phytate–mineral/protein complex. Fish are unable to make use of phytate-nutrient complexes because they lack the enzyme phytase (Riche *et al*., 2001). Tannins hinder the digestive process by binding to digestive enzymes such as protease resulting in a decrease in proteolytic enzyme activity, leading to reduced protein digestibility (Eusebio *et al*., 2004). Tannins also decrease the absorption of essential vitamins (Francis *et al*., 2001). Polyphenols on the other hand, form phenolic-protein-enzyme complexes, these also decrease protein digestibility and amino acid availability. Therefore, the decrease in protein utilisation in fish fed higher levels of MM in the diets is attributed to a decline in the absorption of nutrients. The findings of the current study correspond with those of Bairagi *et al*. (2004) who reported a decrease in growth performance and protein digestibility with increasing leaf meal levels in fish diets.This supports earlier studies which reported that anti-nutritional factors reduce the digestibility and bioavailability of dietary nutrients (Francis *et al*., 2001; Olivera-Novoa *et al*. (2002).

Liver histology indicated an increase in hepatocyte degradation with increasing levels of KM and MM. Fish fed MM had higher degradation scores than those fed KM. *C. gariepinus* fed high MM levels (>50% MM) showed an increase in the number of degraded hepatocytes with irregularly shaped cells, small dark pyknotic nuclei, poor fatty deposition isolated necrosis. In spite of similar protein and energy levels in the experimental diets, liver histology showed that *C. gariepinus* fed higher MM levels had necrotic signs associated with poor nutritional status (Tusche *et al*., 2012; Fontagne *et al*., 1998; Power *et al*., 2000; Ostaszewska *et al*., 2005). The malnutrition signs observed in *C. gariepinus* fed higher levels of MM may be a consequence of the unavailability of protein and amino acids that have been bound with the tannins or have formed indigestible complexes with the polyphenols. As a result of the poor digestibility, a substantial portion of the essential dietary nutrients was not available to the fish and subsequently excreted. This explains the nutritional necrosis observed in the hepatocytes.

Intestine histology in *C. gariepinus* showed significantly shorter villi when fed higher levels of MM in the diet. The longer villi found in fish fed lower levels of KM (<75%) in the diet indicate higher efficiency in the absorptive process (Da Silva *et al*., 2012; Caballero *et al*., 2003). This was evidenced by the better growth performance of fish fed these diets. The decrease in villi height resulted in reduced surface area for nutrient absorption (Da Silva *et al*., 2012); this may also explain the poor condition of the liver. Furthermore, the enterocytes of these fish showed an increase in goblet cell number and microvilli degeneration. The increase in the number of goblet cells may be an indication of increased irritation as these cells produce mucus lining the brush border. This mucus serves as a lubricant providing protection against chemical and mechanical damage. The increase in goblet cell number may also be an immune response against the anti-nutrients (Marchetti *et al*., 2006). Saponins have surface-active constituents which may result in the damage of biological membranes resulting in increased permeability of the mucosal cells (Bureau *et al*., 1998). The saponin content of MM is comparable to that reported in soybean meal up to 83 g kg^{-1} (Ireland *et al*. 1986). The higher saponin concentrations in MM compared to KM may explain the negative effect caused by MM diets in the digestive tract. Bureau *et al*. (1998) reported a reduction in weight gain and significant intestinal damage in Chinook salmon and rainbow trout fed saponin containing diets. It is important to note that dietary inclusion of both leaf meals did not result in some of the intestine abnormalities that have been reported in carnivorous fish fed plant diets. These include a decrease in basophil granulocytes, and distal displacement of enterocyte nucleus (Borquez *et al*., 2011). Other authors have reported a widening of the central stroma within the mucosal folding, higher amounts of connective tissue; and an infiltration of inflammatory cells in the lamina propria (Krogdahl *et al*., 2000; Refstie *et al*., 2000). This may be an indicator that *C. gariepinus* being an omnivore, is more capable of utilising plant diets than carnivorous fish. However, work done on *Tilapia rendalli* (herbivorous fish) fed kikuyu and moringa diets indicate a higher ability of this fish to use plant based diets than *C. gariepinus* (Hlophe and Moyo, 2014). *T. rendalli* had lower levels of hepatocyte and intestine degradation.

Blood parameters are an important tool for monitoring both the nutritional and the health status of fish. In the present study, RBC, Hb and HCT decreased significantly in *C. gariepinus* fed high MM levels. This decrease further indicates nutritional stress. According to Qiang *et al*. (2013) when dietary protein levels are low, physiological stress is induced and this damages the liver, leading to reduced RBC and Hb concentration. Similarly, Sakthivel (1988) and Abdel-Tawwab *et al*. (2010) reported a decrease in

RBC and Hb in fish fed low protein levels in the diet. In spite of the isonitrogenous diets used in this study *C. gariepinus* fed ≥75MM in the diets showed signs of nutritional stress. The WBCs increased with increasing levels of both KM and MM, with higher levels observed in the MM diets. Plasma protein and BUN were significantly lower in *C. gariepinus* fed high MM levels. The low plasma protein observed in *C. gariepinus* may be a consequence of decreased protein absorption emanating from the shorter villi and poor protein digestibility recorded in these fish. BUN represents the amount of nitrogen in the blood that comes from urea. The liver is the main source of urea, thus the lower BUN levels may be an indication of the compromised functioning of the liver.

Cost benefit analysis showed that KM diets are economically superior to both the FM based control and MM diets. Feeding KM diets resulted in higher profit index and lower incidence cost than feeding the FM based control whereas, feeding MM gave lower profit index and a higher incidence cost. Widespread claims on the health benefits in humans have increased the demand and the price for moringa leaves in South Africa, making its use in fish diets unsustainable.

Conclusion

The KM showed greater potential in replacing FM the MM. The growth performance indices (SGR, TGC, FCR, PER) were higher in KM fed fish at all dietary inclusion levels. Histological and haematological analysis indicated that *C. gariepinus* were more stressed as the level of MM in the diet increased. Anti-nutritional factors (polyphenols, tannins, saponins and phytate) are most probably responsible for the poor performance of the moringa diets. It is suggested that the specific roles that each of these anti-nutrients plays in the utilisation of nutrients be further investigated. From the cost benefit analysis it is also evident that kikuyu based diets are more profitable than moringa based diets.

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References

Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A.E. and Shalaby, A.M.E. 2011. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture, 298: 267-274. doi:10.1016/j.aquaculture. 2009.10.027

Afuang, W., Siddhuraju, P. and Becker, K. 2003. Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of moringa (*Moringa oleifera* Lam.) leaves on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research, 34(13): 1147-1159. doi: 10.1046/j.1365-2109.2003. 00920.x

Alarcón, F.J., Moyano, F.J. and Díaz, M. 2002. Evaluation of different protein sources for aquafeeds by an optimised pH-stat system. Journal of the Science of Food and Agriculture, 82: 697-704. doi: 10.1002/ jsfa.1100

Amisah, S., Oteng, M.A. and Ofor, J.K. 2009. Growth performance of the African catfish, *Clarias gariepinus*, fed varying inclusion levels of *Leucaena leucocephala* leaf meal. Journal of Applied Sciences and Environmental Management, 13(1): 21-26. doi:10.4314/jasem.v13i1.55257;

AOAC International. 2012. 19th Edition. Aoac Intl; 19 edition. Association of Analytical Communities. Gaithersburg, MD, USA.

Baeverfjord, G. and Krogdahl, A. 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. Journal of Fish Diseases, 19: 375-387. doi: 10.1046/j.1365-2761.1996.d01-92.x;

Bahnasawy, M.H., Abdel-Baky, T.E. and Gamal, A. 2003. Growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings raised in an earthen pond. Archives of Polish Fisheries, 11: 277-285.

Bairagi, A., Ghosh, K.S., Sen, S.K. and Ray, A.K. 2004. Evaluation of the nutritive value of *Leucaena leucocephala* leaf meal, inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *Labeo rohita* (hamilton) fingerlings. Aquaculture Research, 85: 436-446. doi: 10.1111/j.1365-2109.2004.01028.x;

Bichi, A.H. and Ahmad M.K. 2010. Growth performance and nutrient utilization of African catfish (*Clarias gariepinus*) fed varying dietary levels of processed cassava leaves. Bayero J. of Pure and Applied Sciences, 3(1): 118-122. doi:10.4314/bajopas.v3i1. 58739

Borquez, A., Serrano, E., Dantagnan, P., Carrasco, J. and Hernandez, A. 2011. Feeding high inclusion of whole grain white lupin (*Lupinus albus*) to rainbow trout (*Oncorhynchus mykiss*): effects on growth, nutrient digestibility, liver and intestine histology and muscle fatty acid composition. Aquaculture Research, 42 (8): 1067-1078. doi: 10.1111/j.1365-2109.2010.02690.x;

Bureau, D.P., Harris, A.M. and Cho, C.Y. 1998. The effects of purified alcohol extracts from soy products on feed intake and growth of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 161: 27-43. doi:10.1016/S0044-8486(97)00254-8

Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Montero, D., Socorro, J., Fernández, A.J. and Rosenlund, G. 2003. Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. Aquaculture, 225: 325-340. doi:10.1016/S0044-8486(03)00299-0

Chaudhuri, A., Mukherjee, S. and Homechaudhuri, S. 2012. Diet composition and digestive enzymes activity in carnivorous fishes inhabiting mudflats of Indian

Sundarban estuaries. Turkish Journal of Fisheries and Aquatic Sciences, 12: 265-275. doi: 10.4194/1303-2712-v12_2_11

Cho, C.Y., Slinger, S.J. and Bayley, H.S. 1982. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 73: 25-41. doi: 10.1016/0305-0491(82) 90198-5

Chong, A.S.C., Hashim, R. and Ali, A.B. 2002. Assessment of dry matter and protein digestibility of selected raw ingredients by discus fish (*Symphysodon aequifasciata*) using in vivo and in vitro methods. Aquaculture Nutrition, 8: 229-238. doi: 10.1046/j.1365-2095.2002.00214.x

Collins, S.A., Desai, A.R., Mansfield, G.S., Hill, J.E., Van Kessel, A.G. and Drew, M.D. 2012. The effect of increasing inclusion rates of soybean, pea and canola meals and their protein concentrates on the growth of rainbow trout: Concepts in diet formulation and experimental design for ingredient evaluation. Aquaculture, 344-349: 90-99. doi:10.1016/j. aquaculture.2012.02.018;

Da Silva, M.R., Natali, M.R.M. and Hahn, N.S. 2012. Histology of the digestive tract of *Satanoperca pappaterra* (*Osteichthyes, Cichlidae*). Acta Scientiarum Biological Sciences, 34: 319-326. doi: 10.4025/actascibiolsci.v34i3.8956;

El-Sayed, A.F.M. 2006. Tilapia Culture. CABI Publishers. Oxfordshire, UK, 277 pp. ISBN-13: 978-0851990149;

Eusebio, P.S., Coloso, R.M. and Mamauag, E.P. 2004. Apparent digestibility of selected ingredients in diets for juvenile grouper, *Epinephelus coides* (Hamilton). Aquaculture Research, 35: 1261-1269. doi: 10.1111/j. 1365-2109.2004.01148.x;

Fagbenro, O.A. and Davies, S.J. 2001. Use of soybean flour (dehulled, solvent-extracted soybean) as a fish meal substitute in practical diets for African catfish, *Clarias gariepinus* (Burchell 1822): growth, feed utilization and digestibility. Journal of Applied Ichthyology, 17: 64-69. doi: 10.1046/j.1439-0426.2001.00252.x;

Fasakin, E.A., Balogun, A.M. and Ajayi, O.O. 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. Aquaculture Research, 34: 733-738. doi: 10.1046/j.1365-2109.2003.00876.x;

Fontagne, S., Geurden, I., Escaffre, A.M. and Bergot, P. 1998. Histological changes induced by dietary phospholipids in intestine and liver of common carp (*Cyprinus carpio* L.) larvae. Aquaculture, 161: 213-223. doi:10.1016/S0044-8486(97)00271-8;

Francis, G., Makkar, H.P.S. and Becker, K. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199: 197-227. doi:10.1016/S0044-8486(01)00526-9;

Gatlin, D.B.I.I.I., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R. and Wurtele, E. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquacltutre Research, 38: 551-579. doi: 10.1111/j.1365-2109.2007.01704.x;

Hlophe, S.N. and Moyo, N.A.G. 2014. A comparative study on the use of *Pennisetum clandestinum* and *Moringa oleifera* as protein sources in the diet of the herbivorous *Tilapia rendalli*. Aquaculture Intern., 22: 145-1262. doi: 10.1007/s10499-013-9744-4;

Hlophe, S.N., Moyo, N.A.G. and Ncube, I. 2014. Postprandial changes in pH and enzyme activity from the stomach and intestines of *Tilapia rendalli* (Boulenger, 1897), *Oreochromis mossambicus* (Peters, 1852) and *Clarias gariepinus* (Burchell, 1822). Journal of Applied Ichthyology, 30: 35-41. doi: 10.1111/jai.12290

Hlophe, S.N. and Moyo, N.A.G. 2011. The effect of different plant diets on the growth performance, gastric evacuation rate, and carcass composition of *Tilapia rendalli*. Asian Journal of Animal and Veterinary Advances, 6: 1001-1009. doi:10.3923/ ajava.2011.1001.1009

Ireland, P.A., Dziedzic, S.C. and, Kearsley, M.W. 1986. Saponin content of soya and some commercial soya products by means of high performance liquid chromatography of the sapogenins. Journal of the Science of Food and Agriculture, 37: 694-698. doi: 10.1002/jsfa.2740370715;

Iwama, G.K. and Tautz, A.F. 1981. A simple growth model for salmonids in hatcheries. Canadian Journal of Fisheries and Aquatic Sciences, 38: 649-656. doi: 10.1139/f81-087;

Krogdahl, A., Bakke-Mckellep, A.M., Roed, K.H. and Baeverfjord, G. 2000. Feeding Atlantic salmon *Salmo salar* L. soybean products: effects on disease resistance (furunculosis), and lysozyme and IgM levels in the intestinal mucosa. Aquaculture Nutrition, 6: 77-84. doi: 10.1046/j.1365-2095.2000.00129.x;

Makkar, H.P.S. and Becker K., 1997. Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree. Journal of Agricultural Science, 128: 311-322. doi: 10.1017/S002185969700 4292;

Makkar, H.P.S., Blummel, M., Borowy, N.K. and Becker, K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. Journal of the Science of Food and Agriculture, 61: 161-165. doi: 10.1002/jsfa.27406 10205

Marais, J.P., Figenischou, D.L. and Dennison, C. 1987. The accumulation of nitrate in Kikuyu grass (*Pennisetum clandestinum* Hochst). South African Journal of Plant and Soil, 4: 82-88. doi: 10.1080/02571862.1987. 10634946

Marchetti, L., Capacchietti, M., Sabbieti, M.G., Accili, D., Materazzi, G. and Menghi, G. 2006. Histology and carbohydrate histochemistry of the alimentary canal in the rainbow trout *Oncorhynchus mykiss*. Journal of Fish Biology, 68: 1808-1821. doi: 10.1111/j.0022-1112.2006.01063.x

McFadzen, I.R.B., Coombs, S.H. and Halliday, N.C. 1997. Histological indices of the nutritional condition of sardine, *Sardina pilchardus* (Walbaum) larvae of the north coast of Spain. Journal of Experimental Marine Biology and Ecology, 212: 239-258. doi:10.1016/S0022-0981(96)02755-4;

Olivera-Novoa, M.A., Olivera-Castillo, L. and Martinez-Palacios, C.A. 2002. Sunflower seed meal as a protein source in diets for *Tilapia rendalli* (Boulenger, 1896) fingerlings. Aquaculture Research, 33: 223-229. doi: 10.1046/j.1365-2109.2002.00666.x;

Ostaszewska, T., Dabrowski, K., Palacios, M.E., Olejniczak, M. and Wieczorek, M. 2005. Growth and morphological changes in the digestive tract of

rainbow trout (*Oncorhynchus mykiss*) and pacu (*Piaractus mesopotamicus*) due to casein replacement with soybean proteins. Aquaculture, 245: 273-286. doi:10.1016/j.aquaculture.2004.12.005;

Power, D.M., Melo, J. and Santos, C.R.A. 2000. The effect of food deprivation and refeeding on the liver, thyroid hormones and transthyretin in sea bream. Journal of Fish Biology, 56: 374-387. doi: 10.1111/j.1095-8649. 2000.tb02112.x;

Qiang, J., Yang, H., Wang, H., Kpundeh, M.D. and Xu, P. 2013. Interacting effects of water temperature and dietary protein level on hematological parameters in Nile tilapia juveniles, *Oreochromis niloticus* (L.) and mortality under *Streptococcus iniae* infection. Fish Shellfish Immunology, 34: 8-16. doi: 10.1016/j.fsi. 2012.09.003

Rašković, B.S., Stanković, M.B., Marković, Z.Z. and Poleksić, V.D. 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. Journal of Agricultural Sciences, 56: 87-100. doi: 10.2298/jas1101087r;

Refstie, S., Korsøen, J., Storebakken, T., Baeverfjord, G., Lein, I. and Roem, A.J. 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Aquaculture, 190: 49-63. doi: 10.1016/S0044-8486(00)00382-3;

Riche, M., Trottier, N.L., Ku, P.K. and Garling, D.L. 2001. Apparent digestibility of crude protein and apparent availability of individual amino acids in tilapia (*Oreochromis niloticus*) fed phytase pretreated soybean meal diets. Fish Physiology and Biochemistry, 25: 181-194. doi: 10.1023/A:1022222 431004;

Richter, N., Siddhuraju, P. and Becker, K. 2003. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture, 217: 599-611. doi:10.1016/S0044-8486(02)00497-0;

Sakthivel, M. 1988. Effects of varying dietary protein level on the blood parameters of *Cyprinus carpio*. Proceedings of the Indian National Academy of Sciences: Animal Sciences, 97: 363-366. doi: 10.1007/BF03179546;

SAS 2008, Proprietary software release 9.2. North Carolina, USA. Statistical Analysis Systems Institute, Inc. Cary. http://www.sas.com/en_us/home.html;

Sintayehu, A., Mathies, E., Meyer-Burgdorff, K.-H., Rosenow, H. and Gunther, K.-D. 1996. Apparent digestibilities and growth experiments with tilapia (*Oreochromis niloticus*) fed soybean, cottonseed meal and sunflower seed meal. Journal of Applied Ichthyology, 12: 125-130. doi: 10.1111/j.1439-0426.1996.tb00075.x;

Skelton, P.H. 2001. A Complete Guide of the Freshwater Fishes of Southern Africa. Southern Book Publishers (Pty) Ltd, Halfway House, South Africa, 395 pp.

FAO 2011. Demand and supply of feed ingredients for farmed fish and crustaceans. Trends and prospects. http://www.fao.org/docrep/015/ba0002e/ba0002e.pdf (accessed January 22, 2014)

Tusche, K., Arning, S., Wuertz, S., Susenbeth, A. and Schulz, C. 2012. Wheat gluten and potato protein concentrate-Promising protein sources for organic farming of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 349: 120-125. doi:10.1016/j.aquaculture. 2012.03.009;

Winberg, G.G. 1956. Rate of metabolism and food requirements of fishes. Belorus State University, Minsk. Fish. Res. Bd. Canada, Trans. Ser. No. 194, 1-202. In: J.C. MacKinnon, 1973. Metabolism and its relationship with growth rate of American plaice, *Hippoglossoides platessoides.* Journal of Experimental Marine Biology and Ecology, 11: 297-310. doi:10.1016/0022-0981(73)90029-4;