Effects of selected plant materials on the whole body compositions and hepatosomatic index of Nile tilapia (*Oreochromis niloticus* L.)

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Abstract

An eight week study was carried out to determine the effect of substituting freshwater shrimps *Caridenea niloticus* with 2 plant materials on the whole body composition and the hepatosomatic index in diets used to feed the Nile tilapia *Oreochromis niloticus* in diets. Cassava leaves (CLM) and Boiled tea leaf residues (BTLR) were used to replace freshwater shrimps from diets used to culture the Nile tilapia (*Oreochromis niloticus* L.). The study was carried out in Sagana Aquaculture Centre both in aquaria set up in a hatchery and in hapas set up earthen ponds where 10 post fingerling fish were used in triplicates. The following were used in the experiments: CLM in aquaria (initial mean weight 10.90±0.64g fish^{-1}; mean length 6.20±0.25cm fish^{-1}) and in hapas set up in fertilized earthen ponds (initial mean weight 10.55g fish^{-1} mean length 8.17±0.23cm fish^{-1}) and BTLR in aquaria (initial mean weight 12.36±1.1g fish^{-1}; mean length 8.43±0.27 cm fish^{-1}) and hapas (initial mean weight 10.98±0.75g fish^{-1} and mean length 8.77±0.3 cm fish^{-1}). Four (300g/kg) practical diets were formulated to contain 0%, 25%, 50% or 100% of the test ingredient. The 0% test ingredient (Sagana diet) was used as the control and the performance of fish fed on the other diets were compared to it. The diets were fed to fish in glass aquaria and in hapas in a pond at 10% of their body weight in triplicates. Carcass chemical composition showed similar effect of diets on body moisture content at 25% CLM and 100% CLM in hapas. At 50% CLM the diets caused a significant ($P<0.05$) increase in the whole body moisture level. Substitution up to 50% CLM showed similar effect on body moisture in fish cultured in aquaria. 100% CLM significantly ($P<0.05$) increased whole body moisture, significantly ($P<0.05$) decreased whole body crude protein and significantly decreased Hepatosomatic Indices (HSI) in fish grown in aquaria. HSI was similar at 0% CLM, 50% CLM and 100% CLM but increased significantly ($P<0.05$) in hapas. The diet had the same effect ($P>0.05$) on the whole body chemistry (moisture, total ash and crude protein) in the fish raised in hapas. In glass aquaria total body ash increased ($P<0.05$) significantly with increase in BTLR inclusion. Crude protein decreased significantly at 100% BTLR. The HSI was significantly ($P>0.05$) high at 100% BTLR in hapas, while in the aquaria there was a significant increase in hepatosomatic index with increase in BTLR inclusion. The study concluded that the two ingredients could be used with limited success in aquaria due to their effect on the *O. niloticus*. However the ingredients can be used to replace freshwater shrimps from diets used to culture *O. niloticus* in fertilized earthen ponds.

Key word: *Oreochromis niloticus*, whole body composition, hepatosomatic index

Introduction

Over the last 30 years, aquaculture has grown faster worldwide than any other animal production sector (FAO, 2007). The average annual growth has been 10% compared with 3% in the cattle industry and 1.6% in capture of aquatic species from natural environments (Garduno-Lugo and Olvera-Novoa 2008). The strong growth in aquaculture has generated a consequent 30% annual growth in the production of aquatic species feeds (Francis *et al.*, 2001), and has made raw material supply a continuous challenge in this industry. Tilapia species are used in commercial farming systems in almost 100 countries (Fitzsimmons, 2000). Characteristics that make tilapia a suitable fish for culture include rapid growth rates, high tolerance to low water quality, efficient feed conversion, ease of spawning, resistance to disease and good consumer acceptance (El-Saidy and Gaber, 2005). Rapid growth of tilapia culture has stimulated the expansion of tilapia feed production and a search for novel protein sources to replace animal proteins like fish meal (Marion & Miguel, 2008). Plant or vegetable sources have given a lot of promise as alternatives and equally their availability is assured in most fish farming areas of Kenya. Aquaculture has been slow in its growth in Kenya since its inception. However there has been intervention by the Government of Kenya's (GOK) to increase aquaculture production through the Economic Stimulus Package (ESP) in the 2009/2010 budget-1.1 billion or 8 million/constituency for the creation of 200 fish farming ponds and covering 140 constituencies countrywide. This move targeted, improvement of nutrition, creation of over 120,000 jobs and income opportunities for citizens (Rothuis *et al*., 2011). As is the trend globally where aquaculture growth has generated a consequent 30% annual growth in the production of aquatic species feeds and has made raw material supply a continuous challenge in this industry, the situation in Kenya is that of poor fish quality and quantity. The use of plant sources to replace animal protein has gained a lot of attention in the recent past with relative degree of success. Despite the abudance of plant ingredients, use in feed formulations can be limited because of their antinutritional components, which can be grouped into three categories: (a) those affecting protein utilization and digestion; (b) those affecting mineral utilization; and (c) anti-vitamins and toxic substances (Marion and Miguel, 2008). Investigations on the effects of test diets on the whole body composition and on the Hepato Somatic Indices (HSI) helps explain the biological availability of the ingredients and also effects on the well being of the test fish. This study looked at the possible effects of the diets on the whole body composition and the Hepatosomatic index which can give an indication of lower digestability and poor food absorption respectively.

Materials and methods

The experiment was performed at the Sagana Aquaculture Center 90 Km northeast of Nairobi, altitude 1230 m, latitude 0°39´S and longitude 37°12´E. The experiments were conducted both in laboratory aquaria (dimension 0.45m x 0.3mx 0.3m; water volume, 60 litres), and upper open end hapas dimension of (1m x 1m x 1m) installed in a single earthen pond. The aquaria were set in a thermo regulated recirculating system, comprising a settling tank for solid removal and an anaerobic bio filter (tickling filter) to remove ammonia. Filtered and aerated bore hole water was used in filling the tanks. The experimental pond was fertilized weekly at a rate of 20 kg N and 8 kg P ha-1 with Urea and diammonium phosphate (DAP), respectively, and limed once at 2500 kg ha-1 with $CaCO_3$ at the beginning of the experiment. Key water quality parameters: temperature, pH, dissolved oxygen (DO) and chlorophyll *a* were measured three times a week in the aquaria and cage experiments. Dissolved oxygen was measured using model 57 oxygen meter (YSI industries, Yellow springs, OH, USA), while a glass electrode pH meter, Hi-9024 microcomputer (Hanna Instruments Ltd., Chicago, IL., USA), was used to take pH measurements. Chlorophyll *a* was determined as described in American Public Health Association (APHA, 1995).

Experimental diet formulation and feeding practice

Boiled tea leave residues BTLR were sourced from hotels in Sagana town. Freshwater shrimp meal (FSM) was purchased from Kisumu. Cotton seed cake meal (CSM) and wheat bran were bought from animal feed stores in Sagana town. Cassava leaves were sourced from farms surrounding Sagana Aquaculture Centre. Vitamin and mineral premixes were sourced from an agrovet shop in sagana town. The composition of the vitamin and mineral premixes is shown in Table 5. All ingredients were first dried then ground into fine powder before being subjected to proximate analysis. The proximate composition of the ingredients was determined (as shown in Table 1) for the purposes of formulating the diets. An analysis of crude protein, crude fiber, ether extracts, ash and moisture content was done in triplicates, following the procedure by Association of America Chemists (AOAC, 1995). Protein content of the diets was determined using micro-Kjeldhal method, percent fat using ether extraction method, crude fibre by acid-alkali digestion, ash by burning weighed samples at $600^{o}C$ in a muffle furnace, and moisture by drying samples to constant weight at $100^{o}C$ (AOAC, 1995). Carbohydrate, estimated as nitrogen-free extracts (NFE), was determined by subtracting the sum of crude protein, crude fat, ash and crude fibre from the dry matter content NFE= 100- (% protein+ % fat +% ash + % fibre).

Substitution with boiled teal leaf residues

The ingredients and proximate composition of diets are presented in Table 1. Boiled tea leave residues were used to substitute the freshwater shrimp meal from the control diet. Four isonitrogenous diets were formulated to contain 30% crude protein (CP). The substitutions were made at four levels 0 % (control) 25%, 50%, and 100% of the plant ingredient replacing freshwater shrimps.

Table 1: Proximate composition of ingredients used in formulation of the BTLR diet:

Ingredient	DM	CP	EE	CF	ASH	NFE
BTLR	93.61±0.1	23.97±0.3	23.46±0.41	13.71±0.1	4.76±0.03	35.52±13.3
FSM	93.08±0.1	60.11±0.6	10.31±1.04	6.37±3.3	19.08±0.1	2.79±2.92
CSM	93.56±0.7	23.07±1.3	17.55±0.37	7.40±1.75	19.10±0.1	28.44±2.75
WB	94.43±0.3	19.39±0.7	9.28±0.27	12.95±2.92	3.98±0.1	48.83±2.75

The composition of the experimental diets is shown in Table 2 below. After the ingredients were perfectly mixed, cold water was added with continuous turning over until the mixture became suitable for making granules. The wet mixture was passed through a pelletizing machine. The produced pellets were dried at room temperature for 3 days and then packed in plastic bags until used. Fish were hand fed (as is basis) two times a day at 1000hrs and 1600hrs at 10% of live body weight, weighed once every 2 weeks, and the daily ration adjusted accordingly. Sex reversed *O. niloticus* male fingerlings were used for the feeding experiment. Prior to start of the experiment all fish were acclimatized to the experimental conditions for two weeks and were on the control diet during this period. The diets were allocated to the fingerlings held in the aquaria (initial mean weight 12.36±1.1g fish^{-1}; mean length 8.43±0.27 cm fish^{-1}) and in hapas (initial mean weight 10.98±0.75g fish^{-1} and mean length 8.77±0.3 cm fish^{-1}) in triplicate. The feeding experiment lasted 60 days.

Table 2: Formulation and proximate composition of diets formulated using BTLR as an ingredient .

Diets				
Ingredients	Sagana diet	25%BTLR	50%BTLR	100BTLR
FSM	12	9	6	0
CLM	59	59	59	59
WB	28	28	28	28
BTLR	0	3	6	12
Vitprem	0.5	0.5	0.5	0.5
Minprem	0.5	0.5	0.5	0.5
Total	100	100	100	100
Proximate composition				
Dry matter	92.35±0.30	93.54±0.20	93.14±0.93	93.88±1.27
CP	34.40±0.40	33.81±0.82	31.11±0.40	28.93±0.60
EE	14.59±0.60	14.55±0.68	13.09±0.19	14.25±0.50
CF	6.23±0.90	8.76±0.60	11.36±0.10	12.13±0.47
Ash	8.02±0.17	7.33±0.11	6.92±0.29	4.74±0.28
NFE	27.99±0.83	27.76±0.93	29.98±0.30	50.17±7.4

Substitution with Cassava leaf meal

Diets were allocated to the fingerlings held in the aquaria (initial mean weight 10.90±0.64g fish^{-1}; mean length 6.20±0.25cm fish^{-1}) and in hapas (initial mean weight 10.55g fish^{-1} mean length

8.17±0.23cm fish^{-1}) in triplicate. The feeding experiment lasted 60 days. Each aquarium and hapa was stocked with 10 fish. The proximate values of used ingredients and the proximate composition of formulated diets is shown in Tables 3 and 4 respectively

Table 3 Proximate composition of ingredients used to formulate diets with Cassava leaf Meal

Ingredient	DM	CP	EE	CF	ASH	NFE
CLM	92.80±0.10	23.67±0.10	7.7±0.70	17.1±1.80	8.7±0.10	35.40±3.50
FSM	93.08±0.10	60.11±0.60	10.31±1.04	6.37±3.30	19.08±0.10	2.97±2.92
CSM	95.56±0.70	23.07±1.30	17.55±0.37	7.40±1.75	19.01±0.10	28.44±2.75
WB	94.43±0.30	19.39±0.70	9.28±0.27	12.95±2.92	3.98±0.10	48.83±2.75

Table 4: Formulations and proximate compositions of diets formulated using CLM as an ingredient

Diets				
Ingredients	**Control**	**25%CLM**	**50%CLM**	**100%CLM**
FSM	12	9	6	0
CSM	59	59	59	59
WB	28	28	28	28
CLM	0	3	6	12
VitPrem	0.5	0.5	0.5	0.5
MinPrem	0.5	0.5	0.5	0.5
Total	100	100	100	100
Proximate composition				
Dry Matter	92.35±0.30	89.11±0.93	90.60±0.10	91.10±0.12
CP	34.40±0.40	34.76±1.34	30.63±1.34	30.26±0.44
EE	14.59±0.60	8.05±0.10	6.58±1.55	9.13±1.08
CF	6.23±0.90	10.09±1.21	13.53±4.9	12.13±0.47
Ash	8.02±0.17	4.49±1.61	5.00±0.11	4.96±0.33
NFE	27.99±0.83	32.89±1.73	29.85±1.06	52.48±5.77

Table 5: Composition of vitamin and mineral premixes used in each formulated diets

Vitamin contents		**Mineral contents**	
Vitamin	**Content**	**Mineral**	**Content(mg)**
A	5,000 000UI	Copper sulphate	1.5
D3	1,000 000	Manganese sulphate	90
E	1,500 IU	Manganese iodide	300
B1	600MG	Zinc oxide	70
B2	2500MG	Nicotinic acid	5500
B6	125MG	Calcium pantothenate	5000
B12	75MG		
K	1250MG		

C	200MG		

Whole body chemical composition analysis and hepatosomatic index analysis

At completion of experiment 3 fish per treatment were sacrificed for whole body chemical analysis. The fish were ground in a blender to get a homogenous sample and stored frozen at 20^{o}C. Fish samples were analyzed for proximate composition according to AOAC (1995). The fish were dried at 60^{o}C overnight and ground to determine the dry matter (DM) content. The crude protein (CP) was determined using the micro-Kjeledahel method (Nx6.25) (AOAC, 1995). Ether extract (EE) was determined in soxhalet apparatus using petroleum ether (60-80^{o}C). Ash content was determined in a muffle furnace at 550^{o}C for 3 hours according to AOAC (1995). Three fish per treatment were also dissected carefully to isolate and weigh the liver. HSI was expressed as the relationship between the liver weight and the whole body weight.

Data analysis

Data on fish performance, effect of the diets on the whole body chemical composition and the effect of the diet on the Hepatosopmatic index (HSI) composition was analyzed by single classification analysis of variance (ANOVA) using the methods described by Sokal & Rolhf, (1981). When significance between means was demonstrated, Duncan’s multiple range tests (Duncan, 1955) was used to identify means significantly different from each other. Differences were declared significant at $P \leq 0.05$.

Results

Results of the effects of the diets formulated using BTLR on the HSI and flesh proximate composition are shown in table 6 and 7 respectively. Diets formulated using BTLR had the same effect ($P>0.05$) on the flesh moisture for fish grown both in aquaria and hapas as shown in Table 6 below. The control, 25% BTLR and 50% BTLR treatments had the same effect on the HSI of the fish in hapas. However the HSI was higher ($F= 7.993$ d.f. $=8$ $P< 0.05$) at 100 % BTLR inclusion. In the aquaria, there was a significant difference in the HSI between the 0% BTLR and 25% BTLR and 100% BTLR. However there was no significant difference in the HSI between the 0% BTLR and 50% BTLR for fish grown in the aquaria. In aquaria the diets had the same effect on the flesh crude protein ($F=2.881$, $df=10$, $P>0.05$) at 0%, 25% and 50% BTLR but had a significant decrease at 100% BTLR to record a value of 14.07±0.20%. The diets had the same effect on the flesh moisture content (F=0.923, df=11, P>0.05) in the fish raised in the aquarium. There was a significant (F=23.30, df=8, P<0.05) decrease in flesh total ash with increase in BTLR in aquaria.

Table 6: Effects of the diet on the whole body proximate composition and HSI of *O. niloticus* cultured in aquaria

Levels of substitutions of freshwater shrimps (FSM) with BTLR

Parameter	Control	25% BTLR	50% BTLR	100% BTLR
Moisture	75.50±2.72^{a}	74.45±0.93^{a}	74.98±1.24^{a}	73.04±1.92^{a}
Ash	5.1±0.10^{a}	5.0±0.50^{a}	5.3±0.10^{a}	5.6±0.30^{a}
HIS	0.48±026^{a}	0.71±0.31^{a}	0.44±0.18^{a}	1.53±0.28^{b}

Values are means ± standard deviation of 3 replicates. Means with same superscript are not significantly different at P>0.05

Table 7: Effects of the diet on the whole body proximate composition and HSI of *O. niloticus* cultured in hapas

Levels of substitutions of Freshwater shrimps (FSM) with BTLR

Parameter	**Control**	**25% BTLR**	**50% BTLR**	**100% BTLR**
Moisture	73.57±1.4^{a}	72.94±2.3^{a}	75.07±2.0^{a}	75.45±0.64^{a}
Crude protein	16.16±0.9^{a}	17.18±1.8^{ab}	14.75±0.5^{ab}	14.07±0.20^{b}
Ash	5.3±0.05^{a}	4.9±0.07^{a}	5.8±0.15^{cd}	5.80±0.43^{d}
HIS	0.48±026^{a}	0.71±0.31^{a}	0.44±0.18^{a}	1.53±0.28^{b}

Values are means ± standard deviation of 3 replicates. Means with same superscript are not significantly different at P>0.05

Effects of the diets on the whole body composition and HSI in aquaria is shown in Table 8 and 9 respectively. In hapas there was a significant difference (F=4.083, df=8, P<0.05) in the effect of the diets on whole body moisture of *O. niloticus*. At 25% CLM and 100% CLM the diets had the same effect on the whole body moisture content. At 50% CLM the diets caused a significant increase in the whole body moisture level. In aquaria there was significant difference (F=2.664, df=8, P<0.05) in the effect of the diets on the whole body moisture. Substitution up to 50% CLM caused the same effect on the whole body moisture compared to the control. 100% CLM substitution caused a significant increase in the whole body moisture. In aquaria increase in CLM inclusion in the diets caused a significant (F=8.155, df=8, P<0.05) decrease in whole body crude protein content. In hapas increase in CLM inclusion caused significant decrease in the body HSI. In aquaria increase in CLM beyond 25% CLM caused significantly high HSI (F=2.207, df=8, P<0.05).

Table 8: Effects of the diet on the whole body proximate composition and HSI of *O. niloticus* cultured in aquaria

Levels of substitutions of Freshwater shrimps (FSM) with CLM				
Parameter	**Control**	**25% CLM**	**50% CLM**	**100% CLM**
Moisture	71.23±3.22^{a}	72.48±3.05^{a}	72.16±0.37^{a}	76.53±2.25^{a}
Crude protein	27.82±0.39^{a}	28.04±0.35^{a}	29.88±1.07^{a}	24.67±2.33^{b}
HIS	1.79±0.29^{a}	1.08±0.62^{a}	1.58±0.18^{ab}	0.94±0.49^{b}

Values are means ± standard deviation of 3 replicates. Means with same superscript are not significantly different at P>0.05

Table 9: Effects of the diet on the whole body proximate composition and HSI of *O. niloticus* cultured in aquaria

Levels of substitutions of Freshwater shrimps (FSM) with CLM				
Parameter	**Control**	**25% CLM**	**50% CLM**	**100% CLM**
Moisture	72.97±1.72^{a}	75.34±1.7^{ab}	76.58±0.76^{b}	75.41±0.26^{ab}
HIS	0.51±0.34^{a}	1.65±0.38^{a}	0.85±0.79^{ab}	0.81±0.18^{ab}

Values are means ± standard deviation of 3 replicates. Means with same superscript are not significantly different at P>0.05

Discussion

According to Joachim (2006) plant materials are digested to a lesser degree compared to those of animal origin. This may be attributed to high fibre levels responsible for poor protein digestibility, presence of suppressants, low palatability etc. In our study, diets formulated using BTLR had the same effect on whole body moisture levels in hapas. A similar observation was made in previous studies (Afuang *et al*., 2003) in a study carried out in aquaria to test the suitability of raw and methanol-extracted Moringa *Moringa oleifera* (Lam.) leaf meal to replace 10%, 20%and 30% of the total fishmeal-based dietary protein in tilapia feeds. The study did not find significant effect on whole body moisture with increase in Moringa inclusion in the fish diets. The results however are not similar to Garduno-Lugo and Olvera-Novoa (2008) who reported increased whole body moisture while replacing fish meal with peanut *Arachis hypogeal* leaf meal beyond 20%. The experimental environment was similar to the present study where the fish were raised in a pond. The study also reported a decrease in whole body lipids and ash with increased levels of peanut leaf meal in the diets. In the present study carried out in glass aquaria, the diets did not have a significant effect on the whole body moisture, but an increase in inclusion of dietary BTLR at 100% caused a decrease in whole body crude protein. There was however a decrease in flesh ash for fish in the aquaria with an increase in BTLR inclusion. Moreso analysis done on the ingredients before formulation shows BTLR had less ash content compared to the level in the freshwater shrimps. In aquaria the dietary BTLR caused a decrease in whole body crude protein. A reduced level of whole body crude protein shows a tendency of the fish to convert protein for the purposes of energy production. Low feed uptake in the aquarium may have been a cause for this. There are several reasons which can affect chemical composition of fish flesh. The chemical composition of the flesh can therefore be used to further explain the performance of fish fed on diets formulated using plant materials as ingredients. Despite its high level of crude fibre, Ibrahim and Al-Owafeir and (2004) , incorporated date pits *Phoenix dactylifera* L and their Sprouts in semi-purified diets for Nile Tilapia *O. niloticus* (L.). Ibrahim and Al-Owafeir (2004) concluded that incorporating date pits in semi-purified diets for juvenile tilapia reduced fish growth and negatively influenced the proximate composition of the fish. On the other hand the study found out that substituting corn starch with sprouted date pits at 15% of the diet did not result in a reduction in weight gain or shifts in proximate composition of the fish. In the study, one condition exhibited use of un-sprouted date pits while the other experiment used sprouted date pits. Results showed that inclusion of un-sprouted date pits negatively affected the growth and proximate composition of the fish. This was not experienced in diets with sprouted date pits. The study pointed out the high dietary level of nondigestible carbohydrates in the feed as a likely reason for the poor performance in fish fed on un-sprouted date pits. This was similar to results in other studies where the carp *Cyprinus carpio* L. fed different dietary levels of date pits was used Ibrahim and Al-Owafeir (2004). The authors also showed that total body fat increased and protein decreased as a result of replacement of bran and barley mix (1: 1) with date pits. In a bid to overcome the high levels of high crude fibre content, the study exposed the date pits to sprouting. Ibrahim (2008) suggests that degradedation of date pits using specific enzymes to convert the fibres to simpler forms of carbohydrate molecules may increase utilization of the date pits by animals including fish.

In the present study, increase in CLM replacement of FSM in hapas led to an increase in the whole body moisture content. The results are comparable to those in previous work (Garduno-Lugo and Olvera-Novoa 2008) who reported an increase in the whole body moisture content with an increase in inclusion of peanut leaf meal to replace fish meal from diets used to culture *O. niloticus*. Fish cultured in glass aquaria and fed on 25% CLM and 50% CLM had similar moisture levels as those fed on the control diet. The findings showed a similar trend with those of a previous study (Al-dosari and Belal, 1999) where there was no significant difference in whole body moisture contents when fish meal was replaced up to 40 % from *O. niloticus* diets with sarliconia meal in glass aquaria experiments. The effect was similar on the whole body crude protein values. Cassava leaves replaced up to 50% freshwater shrimps from diets used to culture *O. niloticus* in earthen ponds. In glass aquaria or recirculating systems like concrete tanks cassava leaves can only replace up to 25% of the freshwater shrimp meal. In the glass aquaria increase in inclusion of cassava leaves led to a decline in fish growth performance. Results of our experiment indicate the potential of the test ingredients are limited by their capacity for biological availability.

Several investigation have been done to find out ways of overcoming biological unavailability of ingredients of plant origin for diets formulated for the *O. niloticus.* Ibrahim (2008) found out that by use of a cellulolytic fungus *Trichoderma reesei* that is efficient in producing large amounts of different cellulase-degrading enzymes was one reasons for the high level of growth rates in *O. niloticus* fed on degraded date pits (DPP). The degradation may have been responsible for the increase of digestible carbohydrates (oligo and monosaccarides), which means that protein was not used for the purpose of providing energy for to the fish but rather for flesh development. In addition degradation may have acted to liberate nutrients blocked within date pits fibres which would have led to more digestible nutrients from the DDP. Cao *et al* (2008) carried out pretreatment of diets with microbial phytase to increase phosphorus availability for *O. niloticus* from a plants ingredient. The study investigated effects of pre-treating plant ingredients including soybean meal and a mixed plant meal of soy bean meal, wheat meal and corn glutten. Results showed that addition of phytase and inorganic phosphorus to the basal diet significantly increased the contents of ash, phosphorus and crude protein in the whole body of Nile tilapia, while dry matter and lipid contents decreased significantly. This shows the importance of phytase in making protein available hence increased levels of crude protein in flesh. A trend is observed in the above results that, use of plant proteins or plant based ingredients without prior treatment prevents uptake of important nutrients into the fish body. In results agreeing to the ones mentioned above Asraf *et al*., 2007 investigated the growth performance and feed utilization of Nile tilapia *O. niloticus* Linnaeus and tilapia *Galilae sarotherodon galilaeus* L fingerlings fed plant protein-based diets. The work shows that feeding fish with corn glutten meal increased the values of whole body crude proteins and lipids. However in the same study the lowest ash content was observed with diet containing extruded full fat soyabean meal. The study points out the reduction of ash content can be as a result of presence of phytic acid which reduces the availability of several minerals like calcium, magnesium, zinc, iron and phosphorus. Mohsen and Mohammad (2009a) investigated use of live Spirulina (*Arthrospira platensis*) as a growth and immunity promoter for, *Oreochromis niloticus* L., challenged with pathogenic *Aeromonas hydrophila.* Results showed that Spirulina had a growth promoting influence on the *O. niloticus* as was seen in optimum growth and feed utilization. Improved whole body protein and lipid content were also related to the positive effects of the Spirulina.

Management of dietary protein during the growing period on growth performance, feed utilization and whole-body chemical composition of *O. niloticus* L. has also been investigated (Mohsen and Mohammad 2009b). In this study the contents of moisture, crude protein and total lipids in whole-fish body were not significantly affected by protein management except ash content, which was higher in the fish. The study suggested that nutrient digestibility and deposition may not have been affected by protein management but they also added that protein and lipid contents in fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rates. Feeding rate can also have an impact on the proximate body composition (Jamjun and Amararatne, 2005). Investigations show that body lipid increased as well as moisture and ash percentages decreased with increased feeding rate. Studies have also shown that there was a decrease in ash content when the body lipid content increased at higher feeding levels of *O. niloticus.* Equally body protein content of experimental fish increases with increase in feeding rate (Jamjun and Amararatne, 2005).

In addition to the methods used to improve biological availability of the nutrients studies have also been carried out to show the importance of the encouraging natural feed production in earthen ponds. Most farming of *O. niloticus* is done in earthen ponds. Use of formulated feeds incorporating plant ingredients can only be successful under proper earthen pond conditions. A study by Ali and Mohamed (2002) sheds light on the importance of natural feed resources in earthen ponds. In their study, effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds are investigated. Increased feeding rates had either no effect or irregular effects on the percentages of protein and ash gains in the fish body. Notably there was significant increase in percentages of fat and gross energy gains accompanying linear decreases in percentages of moisture. The study related the increase in percentage of fat gains in the body to increased ingestion. Unfed fish were found to have the lowest percentage of fat and highest percentage of moisture in their flesh. This may have been as a result of low fat and low energy contents of the pond natural food and organisms. Natural food organisms contain low energy but are rich in protein (Ali and Mohamed, 2002), therefore fish consuming only natural food have minimal fat and maximal protein accumulation in their bodies. Supplemental feeding showed high percentages of fat in their bodies and lowest moisture gain. However Azim *et al.*, 2003 found no effects of density or substrate on proximate composition of fish when they investigate the effects of periphyton substrate and fish stocking density on water quality, phytoplankton, periphyton and fish growth. In this study the *O. niloticus* at harvest had their ash content slightly higher while protein content was slightly lower. A study by Mario and Miguel (2008) showed a change in body composition with replacement of fishmeal with peanut leaf meal. The changes are noted with marked increased levels of peanut leaf meal. The study showed an increase in moisture content at 30% Peanut Leaf Meal (PLM) inclusion however the moisture was not higher than the moisture in fish at the beginning of the experiment. The study also found similar results as those of previous study as with protein, fat and lipid levels. This study argues that protein, fat and lipid levels decline with increase in plant protein replacement levels due to lower digestibility and consequent lower nutrient availability in the diets with high plant protein levels. The study by Mario and Miguel (2008) however concluded that the diets formulated using peanut leaf meal had insignificant impact on the growth of the *O. niloticus*, and suggested that the pond environment probably had a positive effect on the growth of fish. The study described different culture conditions as those used in the present study where static ponds were used in comparison to recirculating ponds used by Mario and Miguel (2008). Though their study concluded that up to 20% substitution had no negative effects on growth of *O. niloticus*, the study recommends further research on the economic evaluation to determine the impact of the substitution on the production costs.

The HSI of fish fed the BTLR-containing diets were noticeably the same but increased significantly at 100%BTLR, as can be observed from the HSIs in hapas. The current study can not explain the increase in HSI at 100% BTLR. However a possible lipid deposition in the liver may have been possible leading to increase in the liver weights as a nutrient storage mechanism. Afuang *et al*., (2003) associated lower nutrient availability and feed intake to low nutrient storage hence linking it to reduced liver sizes.

Conclusion

The present study equally concludes that boiled tea leaf residues (BTLR) that are normally thrown from kitchens as wastes can actually be used to substitute up to 50% of freshwater shrimps from diets used to culture *O. niloticus*. In an intensive indoor production systems replacement is impossible and led to decrease in growth performance with increase in inclusion level. BTLR can therefore replace FSM in *O. niloticus* diets without negative effects on the whole body composition or the HSI. On the other hand cassava leaves can replace up to 50% freshwater shrimps from diets used to culture *O. niloticus* in earthen ponds. In glass aquaria or recirculating systems like concrete tanks, cassava leaves can only replace up to 25% of the freshwater shrimp meal. The study recommends for an economic evaluation study to determine the impact of the substitution on the production costs for effective inclusion in *O. niloticus* production at small scale level. It is also recommended that trials should be done using pretreated BTLR and CLM to find out their potential in diets for *O. niloticus*.

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