Global Journal of Science Frontier Research
Chemistry
Volume 13 Issue 2 Version 1.0 Year 2013
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Chemical Analysis and Nutritional Assessment of Defatted Garcinia Mangostana Seeds Used as an Additive on the Feed of Fish *(Clarias Gariepinus)*

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GJSFR-B Classification : *FOR Code : 630503, 090803*

Strictly as per the compliance and regulations of :

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I. Introduction

Uncountable plant resources with important benefits abound around us unexploited. Many have high nutritive values like proteins, carbohydrates, lipids and minerals while others have industrial potentials as sources of dyes, starches and vitamins. Plants hitherto considered of little or no value are being investigated, evaluated and developed into drugs with little or no side effects (Adedeji et al., 2006). Several biological and synthetic compounds have been shown to enhance non specific immune system of cultivated fish (Cao & Lin, 2003; Lin & Zhang, 2004; Sakai, 1999; Shan et al, 1999; Soosean et al. 2010). Lack of competition for and high cost of readily available nutritive fish feed ingredients have continued to plague aquaculture in the competitive global food production system (Munguti et al., 2012; Tacon, 1993). In view of these, researchers have been studying several plant and animal sources as supplement or likely substitutes for the conventional ingredients currently in use. Several studies have shown that vegetable protein sources have high potentials for supplying fish with required protein needed for their maximum productivity (Nwanna et al.., 2008). Protein is the most expensive component of fish diets and is usually supplied as fish meal due to its balanced amino acid profile (Munguti et al, 2012). As alternatives to fishmeal, plant products tend to be less balanced in amino acid profiles than fish meal. Omoregie and Ogbemudia (1993) advised that it would be more economical to use plant protein and have reduced growth rates of aquatic animals than using fish meal at high cost.

Feed additives are substances which are usually included in feeds in trace amounts to preserve its nutritional characteristics prior to feeding (antioxidants and mould inhibitors), facilitate ingredient dispersion or feed pelleting (emulsifiers, binders or stabilizers), promote growth (growth promoters, antibiotics and hormones), facilitate feed intake and acceptance (feeding stimulants and colourants), or supply some essential nutrients such as minerals and vitamins (Food and Agricultural Organization, 1987).Some workers have also suggested the addition of immunostimulants of biological origin to reduce disease outbreaks in fish culture by enhancing the non-specific immune system (Anderson,1992).

The Genus *Garcinia* belongs to the family *Clusiaceae* (syn. *Guttiferae*) which consists of about 35 genera and up to 800 species (Osman & Milan, 2006). The family is pan tropical and comprises mostly of large evergreen trees or erect shrubs with smooth, thin bark and white or yellow latex. *Garcinia* is the biggest Genus in the family with about 400 species (Jantan et al., 2011). The Genus *Garcinia* has been used in ayurvedic preparations to treat disorders and contain bioactive

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molecules like hydroxycitric acid, flavonoids, terpenes, polysaccharides, procyanidines and polyisoprenylated benzophenone derivatives like garinol, xanthochymol and guttiferone (Hemshekhar et al., 2011; Lim, 2012). Flavonoids present in G. *kola* are assumed to have anti-asthmatic activities (Okojie et al., 2009), inhibit platelet activating factor, phospholipidaseA2 and phosphodiesterase (Dorsch & Wagner, 1991; Miller, 2001), prevent allergies, inflammation, free radicals, platelet aggregation and inhibit histamine release (Ferguson, 2002; Farquar, 1996; Hodek et al., 2002; Okwu, 2004). *Garcinia mangostana* L., (mangosteen) is a slow-growing tree and well known for its fruits particularly in Southeast Asia. The fruits possess a sweet pulp which is eaten fresh, but is also used in processed form. It has a long history of use as a medicinal plant majorly in Southeast Asia for its anti inflammatory properties (Obolskiy et al., 2009) and for treatment of skin infections and wounds (Pedraza-Chaverri et al., 2008). Other applications include treatment of dysentery, urinary disorders, cystitis and gonorrhea.

The biologically active ingredients in G mangostana L. responsible for these medicinal properties have been identified as xanthones (Obolskiy et al., 2009) which are polyphenols found in the pericarp from the mangosteen fruit .The juice also contains α-mangostin, garcinones C, D, and E, γ-mangostin, gartanins and other xanthones (Chitchumroonchokchai et al., 2012; Pedraza-Chaverri et al., 2008;). α-mangostin has been shown to enhance betulinic acid's cytotoxicity to colon cancer cells, showed cytoprotective effect against cisplastin-induced cytotoxicity (Aisha et al., 2012) and is a multi-target inhibitor of mutans streptococci and useful as an anti-caries agent (Phuong et al., 2011). Poly isoprenylated benzophenone and xanthone are known for having antioxidant (Joseph et al., 2005; Okoko, 2009), antibacterial, anti viral, anti fungal, anti ulcer, anti protozoan activities (Hemshekhar et al., 2011), anti platelet (Jantan et al., 2009) and anti-cancer (Han et al., 2008). Ajayi et al., (2007) observed that rats were able to utilize the oil of *Garcinia mangostana* seeds and suggested that the seeds could be utilized as sources of dietary fibre and roughage in livestock feeds because of its high crude fibre and carbohydrate contents. The low protein content can be supplemented with high protein residues, such as groundnut or soy cakes. A lot of studies using G. *mangostana* for treatment of human disorders abound including heart problems and asthma (Buelna-Chontal et al, 2011; Okojie, et al., 2009). This study examined the utilization and growth performance, haematology and tissue pathology of *Clarias gariepinus* post juveniles on feeds containing defatted G. *mangostana* seed meal included as additives of 0.00g, 18.00g, 36.00g, 54.00g and 72.00g.

II. Materials and Methods

a) Sample Preparation

Garcinia mangostana fruits were obtained from the Botanical Garden of the University of Ibadan. The seeds were removed from the fruits, washed with water and left to air-dry. Oil from the seeds was obtained by soxhlet extraction using n-hexane (bpt 60 °C) as described by Ajayi et al. (2006) and left to air dry for a week to remove the solvent from the defatted seeds.

b) Proximate Analysis

Proximate analysis of the defatted seeds, formulated feeds and fish samples were carried out following the procedure described by AOAC (2000).

c) Mineral element analysis

The mineral element analysis was carried out using the wet-ashing method for the digestion of the defatted seed sample: 1.00g of defatted G. *mangostana* seed was digested with 20 ml of concentrated HNO^3 and perchloric acid (1:1 v/v) and thereafter transferred to a 50 ml volumetric flask. It was diluted to volume with de-ionized water and stored in a clean polyethylene bottle. The mineral element content was determined using an atomic absorption spectrophotometer (Perkin–Elmer model 703, USA) as described by Onyeike and Acheru (2002).

d) Fish housing and treatment

Clarias gariepinus post juveniles (One hundred and fifty, initial mean length and weight 16.20 ± 0.01cm and 47.92 ± 0.00g respectively) were purchased and transported from the Department of Wildlife and Fisheries Management's fish farm, University of Ibadan, Oyo State to the laboratory in plastic bowls between 5pm-6pm to reduce stress and mortality due to high temperature. The fish were acclimatized for five days during which vitamin C was administered in water. The water in the bowls was well aerated using air stone and pump (Lawson, 1995) and fish were fed at 3% of their body weight twice daily. At the end of acclimatization period, the fish were starved for 12 hours to empty their guts and prepare them for the experimental feed.

The fish were assigned randomly to five treatments in 40- litre plastic bowls with each treatment having three replicates and 10 fish per replicate. De-chlorinated water (tap water exposed to air for more than 24 hours) was used. Water quality parameters monitored were temperature using mercury- in- glass thermometer (Paragon Scientific Ltd, Birkenhead, and Wirral, UK). The dissolved oxygen content and pH of the water were measured at 0.01 accuracy using dissolved oxygen metre, (Jenway 3015 pH metre, Genway, Staffordshire, UK) after standardizing the metre. These water quality characteristics were monitored throughout the feeding trials and water in each bowl was changed every three days. Fish were fed experimental diets twice daily for 49 days at 3 % of

their body weight. Their weights and lengths were recorded weekly. The plastic bowls were covered with nets to prevent the fish jumping out and avoid intrusion by other animals and insects.

e) Feed Formulation

The feedstuffs purchased from the feed mill were soybean meal (44% crude protein), fish meal (72 % crude protein), wheat offal (18 % CP), starch, di-calcium phosphate (DCP), salt, maize, vegetable oil and vitamin/mineral premix. The ingredients were milled into powder and used to formulate a 40 % crude protein diet and turned into dough. Each diet mixture was treated separately and extruded through a 1/4mm die mincer of Hobart A-200T pelleting machine (Hobart GmbH, Rben-Bosch, Offenburg, Germany) to form noodle- like strands which were broken into suitable sizes for the fish. The pellets were sun- drieds, packed in labeled polythene bags and stored in a cool dry place to prevent fungal growth. The defatted *Garcinia mangostana* (DGMS) was incorporated in the formulated diet as an additive at 0.00g, 18.00g, 36.00g, 54.00g, 72.00g inclusion levels before the extrusion process representing diets 1(control) to 5 respectively as shown in table 1.

f) Food utilization parameters

Specific growth rate (SGR): This was calculated from data on changes of the body weight over the given time intervals according to the method of Brown (1957) as follows:

$$SGR\% = \frac{\text{Ln } W2 - \text{Ln } W1}{T - t} \text{ X } 100$$

Where W1 is the initial weight (gram at time t), W2 is the final weight gain (gram at time t) (Brown, 1957)

i. *Food conversion ratio*

$$FCR = \frac{\text{weight of food consumed fortnightly (g)}}{\text{weight gain by fish fortnightly (g)}}$$

Weight gain (g) was calculated as the difference between the initial and final mean weights of the fish in the plastic bowl.

weight gain(g) = final weight-initial weight

Survival rate (SR): The survival rate, SR was calculated as total fish number harvested / total fish number stocked expressed in percentage.

$$\text{survival (\%)} = \frac{\text{number of fish harvested}}{\text{number of fish stocked}} \text{ X } 100$$

Percentage weight gain (%WG): This is expressed by the equation:

$$\%WG = \frac{Wt - Wo}{Wo} \text{ X } 100$$

Where: Wo = Initial weight, and Wt = Weight at time t.

Protein efficiency ratio (PER): This was calculated as:

$$PER = \frac{\text{wet body weight gain}}{\text{crude protein feed}}$$

Condition factor (K): This was expressed by the equation

$$K = \frac{100\,W}{L^3}$$

Where W is weight of the fish
L is standard length
Nitrogen metabolism (NM) was calculated as:

$$NM = \frac{(0.549)(a+b)h}{2}$$

Where a = initial weight of fish
b = final weight of fish
h = experimental periods in days (Nwanna, 2003).

g) Heamatological Analysis

Hematological analyses were carried out both at the beginning and end of the experiment. Initial and final fish blood samples were collected before the feeding trial (that is day 10) and on day 49 for all treatments with the aid of needles and syringes into heparinised bottles with disodium EDTA as anticoagulant. The samples collected were analyzed on each occasion as described by Schalm et al., (1975). The blood parameters determined for each sample were packed cell volume (PCV), haemoglobin concentration, white blood cell (leucocyte) count, red blood cells (erythrocyte) count, protein, albumin, and globulin. Others were mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC).

h) Histopathological Analysis

For tissue histopathology, the internal organs were exposed by dissection and the liver, kidney, heart, brain were observed for gross lesion and stored in formalin. Small portions of each organ were fixed and put through series of dehydration in graded concentrations of xylene. They were embedded in wax, sectioned at 5µ and transferred onto glass slides. The thin sections were stained with heamotoxylin and eosin (H and E) dyes for examination under the light microscope for histological changes (MAFF, 1984).

i) Statistical Analysis

Each experiment was performed in triplicate. Results were expressed as the mean ± standard deviation. The one-way analysis of variance (ANOVA) was used to determine significant differences of the treatments. P-values<0.05 were considered significant. Duncan multiple range test was applied while all data

were analyzed with SPSS (IBM statistic Computer Program 2010).

III. Results and Discussion

The proximate composition of *G. mangostana* defatted seed was presented in able 2 and showed the protein content of the defatted seed as low and similar to quantities obtained from some cereal grains like maize (8-9%). The crude protein for *G. mangostana* obtained during this study was higher than 6.57 g/100g reported by Ajayi et al. (2007) but lower than for *Gnetum africanum* 17.50% (Ekop, 2007). The crude fibre content (6.50 ± 0.02 %) was higher than the range for legumes (Prakash et al. 2001). Carbohydrate content was high at 71%.This implies that the seeds can complement energy sources or supply energy in livestock rations. The ash content was 1.80 ± 0.02% and similar to Ajayi et al. (2007) but lower than 4.5% reported for *Caesalpinia pulcherrima* (Yusuf et al. (2007). The crude fat content (2.13 ± 0.04) is quite low when compared with that of Ajayi et al. (2007). The low fat content of the defatted seed reported was because of the removal of the oil by solvent extraction during the defatting process.

The defatted seeds of *G.mangostana* had a good supply of mineral elements (Table 6) with the highest being potassium (270 ppm), followed by magnesium (110 ppm), iron (68.62 ppm) and calcium (30 ppm). A diet that contains *Garcinia mangostana* will help to prevent deficiencies of potassium, iron and calcium since they are rich in these elements. Potassium helps to regulate blood pressure; calcium is needed for good bones, muscle contraction and blood clotting while magnesium works along with calcium to maintain healthy bones. The minerals obtained in the defatted seeds of G. mangostana were lower than those obtained in the seed oil (Ajayi et al., 2007).This may imply that most of the mineral elements were lost to the oil fraction during the defatting process.

The gross and proximate compositions of the diets were presented in tables 1 and 2 respectively. All diets were formulated with similar gross compositions except for the addition of 0.00, 18.00, 36.00, 54.00 and 72.00g of the defatted *G.mangostana* seed to the experimental diets for diets 1-5 respectively. Though the moisture contents of all the diets were significantly different ($p<0.05$), the crude protein of the experimental diets were not. Crude fat (ether extract), crude fibre, ash and Nitrogen free extractives varied significantly among treatments ($P<0.05$).These could have been due to the presence of DGM at different levels in the diets.

Growth and nutrient utilization of *Clarias gariepinus* on the experimental diets were presented (Table 3). Fish on all treatments significantly ($p<0.05$) increased in weights and lengths over the experimental period above the initial observations though no significant differences ($p<0.05$) were observed in weights and lengths among treatments. Feed conversion ratio, specific growth rate, percentage weight gain, and nitrogen metabolism in fish did not differ significantly among the diets. This reveals that the addition of defatted seeds of *G. mangostana* as an additive to the feed of *C. gariepinus* had a positive effect on the growth performance of the fish throughout the experimental period. These observations were similar to those observed by (Dada & Oviawe, 2011, Prasad & Priyanka, 2011; Dada & Ikuerewo 2009) but differed from Soosean et al. (2010) .Higher survival rates were recorded in DGM-containing diets above the control with the highest in diet 5(72.00g DGM). The condition factors of the fish varied non- significantly in all treatments. The feed conversion ratios for diets 2, 3 and 4 were lower than for the control and treatment 5 showing that diets containing between 18.00g DGM and54.00g DGM were better utilized by the fish for growth than the control and highest inclusion level of DGM though the observed differences were not significantly different ($p>0.05$).

The proximate analyses of *C.gariepinus* post juveniles after the feeding trial were presented in table 2. Moisture contents, crude protein and crude fibre of the fish differed significantly ($P<0.05$) among treatments. The crude protein was highest in 18.00g DGM-containing diet while the crude fibre was highest in 36.00g DGM-containing diet. The crude fat (ether extract and ash were not significantly different among treatments and varied from 20.30±0.27 to 23.70±1.67. All the DGM-containing diets had higher ash contents than the control fish with diet 3(36.00g DGM) having the highest value (10.60 ± 1.96) and the lowest in the control and 18.00g DGM diet. This observation tends to support the view that DGM-containing rations could improve the mineral content of the fish. The mineral composition of *Clarias gariepinus* (Table 6) after the feeding trial showed significant differences ($p<0.05$) among treatments in potassium, calcium, magnesium and sodium which are macro elements with diet 4(54.00g DGM) producing the highest concentrations of the elements and diets 1 and 2(18.00g DGM) the lowest of all four in the fish. Manganese, iron, copper and zinc were present in very low quantities and were not significantly different among diets.

All the blood parameters studied were higher after 49 days than at the beginning of the study except heterophils, MCH and MCV which decreased from initial values (Table 4).These observations were similar to the reports of other workers (Prasad & Priyanka, 2011; Soosean et al, 2010) who reported increased haematological parameters in fish after diets containing different extracts of Garcinia species. Packed cell volume varied from 30.00 ± 2.83 to 34.00 ± 1.41% for all the treatments though not significantly different ($p>0.05$). Haemoglobin concentration (mg/l) of the fish in all treatments and control varied but not significantly. The hemoglobin content in the blood and oxygen

consumption increases when fishes are stressed and allows an increase in release of immature RBCs from the haemopoietic organs, which in turn elevate hemoglobin concentration in blood (Choudhury et al, 2005; Soosean et al, 2010).

White blood cell counts in all the treatments were significantly higher than the initial values and differed significantly among the treatments. This supports the immune- system boosting effect of Garcinia species reported by some workers (Dada & Ikuerewo, 2009; Prasad & Priyanka, 2011). The platelets also showed similar trend. Percentage lymphocytes and monocytes varied non-significantly among diets from 71.00±1.41 to 72.00 ± 2.83% and 2.00 ± 1.41 to 4.00 ± 1.41respectively. The survival rate of the fish was high generally during the experiment though the least was reported for the contro

The blood biochemistry -plasma proteins (albumin and globulin) and blood serum enzymes (aspartate amino transferase and alanine amino transferase) were presented in table 5. All the final mean values were higher than the initial observations except for aspartate amino transferase (AST) in which the mean value for fish on control diet was lower than the initial observations .However all DGM-containing diets produced higher AST at 49 days than at day 0 of the experiment. Total protein ranged from 3.50±1.4 to 3.80 ± 0.14 and albumin 1.10 ± 0.14 to 1.40 ± 0.14 for all the diets. The highest value of globulin was 2.8 ± 1.4 in diet 3(36.00g DGM) and the lowest 2.20 ± 0.14 in diet 2(18.00g DGM). The values of AGR (albumin –globulin ratio) were not significantly different, ranging from 0.30 ± 0.14 to 0.60 ± 0.14 for all diets. The blood biochemistry showed variations that were not significant ($p>0.05$). Fish plasma proteins are important in regulating water balance in fish (Wedemeyer & Yasutake, 1977)

The tissue examination showed no lesions in the organs of the control fish. Other diets produced no lesions in the organs except the gills in diet 2(36.00g DGM) fish which showed mild submucosal congestion. Diet 5(72.00g DGM) produced severe interstitial congestion in the kidney. The kidney is involved with excretion of wastes from the body of animals and that could explain why there was severe interstitial congestion at the highest inclusion level of DGM inclusion treatment 5 (72.00g DGM). Similar reports of kidney malformations were reported on rats (Ajayi et al, 2007). The organ majorly impacted in *C.gariepinus* post juveniles was the liver which presented different degrees of hepatocyte vacuolation in diets 2, 3 and 5(18.00, 36.00 and 72.00g DGM). Diet 4 (54.00gDGM) was the only group where the fish showed no visible lesions in all organs in fish on DGM-containing diets. This observation differed from Ajayi et al. (2007) who reported no lesions in the liver of rats. The liver is the organ involved in the metabolism, detoxification and excretion of chemicals and xenobiotics in the body (Pathan et al, 2010) and that could explain why it was so affected. Vacuolation has been observed to be a common response to the presence of chemicals in fish (Meyer & Henderick, 1985; Clearwater et al, 2002; Shaw & Handy, 2006). No lesions were observed in all the heart tissues of fish during this study.

IV. Conclusion

The proximate analysis of the defatted *Garcinia mangostana* seed (DGM) depicted it as high in carbohydrate and good in mineral elements though the protein content was low. The mineral analysis of defatted *Garcinia mangostana* seeds showed high values of potassium, magnesium and iron which indicated that DGM could be used as an additive in fish feed. However, further studies are recommended due to the problems identified during the tissue examination of the liver. High survival rate and good performance of the fish would be an incentive to further look at methods of processing the DGM to be compatible with the requirements of *C.gariepinus*.

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Table 1 : Gross composition of experimental diets (g) containing defatted G. mangostana seeds

Ingredients(g)	Control	18gDGM	36g DGM	54gDGM	72gDGM
Fish meal	9.69	9.69	9.69	9.69	9.69
Soy bean	19.39	19.39	19.39	19.39	19.39
Maize	31.71	31.71	31.71	31.71	31.71
Wheat offal	31.71	31.71	31.71	31.71	31.71
Vit-min	2.00	2.00	2.00	2.00	2.00
Starch	1.00	1.00	1.00	1.00	1.00
DCP	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50
Vegetable oil	2.00	2.00	2.00	2.00	2.00
DGM(g)	-	18.00	36.00	54.00	72.00

Table 2 : Proximate composition of formulated diets at different inclusion levels of defatted *G. mangostana* seeds

Constituents(%)	Control	18g DGM	36g DGM	54g DGM	72g DGM
Moisture	6.64 ± 0.045^{d}	6.07 ± 0.061^{b}	5.68 ± 0.03^{a}	6.31 ± 0.01^{c}	7.60 ± 0.02^{e}
Crude protein	40.39 ± 0.40^{a}	40.49 ± 0.22^{a}	40.36 ± 0.25^{a}	40.21 ± 0.01^{a}	40.96 ± 0.01^{a}
Crude fibre	4.82 ± 0.15^{b}	3.53 ± 0.01^{a}	3.49 ± 0.02^{a}	5.53 ± 0.01^{c}	5.77 ± 0.08^{d}
Crude fat	7.70 ± 0.01^{c}	7.07 ± 0.05^{a}	7.14 ± 0.01^{b}	15.60 ± 0.32^{e}	15.11 ± 0.04^{d}
Ash	8.45 ± 0.27^{b}	8.31 ± 0.16^{b}	8.2 ± 0.01^{ab}	8.00 ± 0.03^{a}	7.95 ± 0.14^{a}
NFE	32.00 ± 0.80^{c}	34.52 ± 0.47^{d}	35.33 ± 0.26^{e}	24.35 ± 0.01^{b}	22.62 ± 0.13^{a}

Means followed by different letters in the same rows are significantly different ($P<0.05$)

Table 3 : Result of proximate analysis of fish after feeding trial using experimental feeds

Constituent (%)		Mean ± SD				
	Garcinia mangostana defatted seeds	Control	18.00 g DGM	36.00g DGM	54.00g DGM	72.00g DGM
Moisture	10.47 ± 1.12	9.48 ± 0.55^{a}	9.08 ± 0.99^{b}	9.92 ± 0.09^{a}	10.46 ± 0.58^{c}	9.48 ± 0.55^{b}
Crude protein	8.10 ± 0.22	63.00 ± 2.00^{cd}	63.60 ± 0.26^{d}	55.40 ± 0.27^{a}	60.70 ± 0.34^{b}	61.47 ± 1.27^{bc}
Crude fibre	6.50 ± 0.02	0.27 ± 0.06^{a}	0.23 ± 0.06^{a}	0.200 ± 0.00^{a}	0.40 ± 0.10^{b}	0.70 ± 0.00^{c}
Crude fat	1.80 ± 0.02	20.60 ± 1.85^{a}	20.30 ± 0.27^{a}	23.70 ± 1.67^{b}	21.07 ± 0.78^{a}	20.50 ± 0.46^{a}
Ash	2.13 ± 0.04	6.65 ± 0.55^{a}	6.79 ± 1.83^{c}	10.60 ± 1.98^{b}	7.37 ± 0.22^{b}	7.85 ± 0.55^{b}
Carbohydrate	71.00 ± 0.79					

Means followed by different letters in the same rows are significantly different ($P<0.05$).
The treatments produced very negligible nitrogen free extracts in fish

Table 4 : Mineral composition of Clarias gariepinus post juveniles after feeding trial (ppm of dry matter) in 49 days and Defatted *Garcina mangostana*

Mineral element	Defatted *Garcinia mangostana*	Control	18.00g DGM	36.00g DGM	54.00g DGM	72.00g DGM
Potassium	270	73.30^{b}	32.80^{a}	249.50^{d}	364.50^{e}	147.00^{c}

Calcium	30	160.00^{b}	120.00^{a}	258.00^{d}	268.50^{e}	242.00^{c}
Magnesium	110	12.50^{b}	6.87^{a}	35.65^{d}	49.65^{e}	23.75^{c}
Sodium	7.95	50.41^{b}	20.11^{a}	147.05^{d}	134.05^{e}	58.31^{c}
Manganese	9.76	0.12^{a}	0.12^{a}	0.30^{a}	0.38^{a}	0.37^{a}
Iron	68.62	1.85^{a}	1.88^{a}	4.42^{b}	4.27^{b}	1.95^{a}
Copper	6.58	0.10^{a}	0.02^{a}	0.09^{a}	0.16^{a}	0.13^{a}
Zinc	25.63	0.50^{a}	0.14^{a}	0.87^{a}	1.50^{a}	0.69^{a}

Table 5 : Growth and nutrient utilization of Clarias gariepinus fed with different defatted G. mangostana-based diets

Parameter	Control	18.00g DGM	36.00g DGM	54.00g DGM	72.00gDGM
IMW	47.89±0.11^{a}	47.92±0.06^{a}	47.89±0.11^{a}	47.93±0.17^{a}	48.00±0.19^{a}
FMW	56.16±4.23^{a}	59.06±1.22^{a}	58.31±2.19^{a}	56.75±3.84^{a}	55.44±1.42^{a}
PER	0.25±0.12^{a}	0.33±0.04^{a}	0.33±0.07^{a}	0.29±0.12^{a}	0.30±0.06^{a}
CF	0.58±0.01^{a}	0.67±0.04^{ab}	0.70±0.03^{b}	0.65±0.10^{ab}	0.59±0.07^{ab}
FCR	2.15±0.92^{a}	1.46±0.14^{a}	1.55±0.28^{a}	1.94±0.78^{a}	2.05±0.33^{a}
NM	1399.58±55.55^{a}	1439.07±17.12^{a}	1428.44±30.86^{a}	1407.95±53.90^{a}	1391.37±20.85^{a}
WG	8.27±4.33^{a}	11.14±1.16^{a}	10.42±2.09^{a}	8.82±3.67^{a}	7.44±1.31^{a}
SGR	0.32±0.16^{a}	0.43±0.04^{a}	0.40±0.07^{a}	0.34±0.13^{a}	0.29±0.05^{a}
PWG	17.29±9.10^{a}	23.24±2.40^{a}	21.75±4.31^{a}	18.39±7.59^{a}	15.50±2.68^{a}
IL	16.20±0.00	16.20±0.00	16.20±0.00	16.20±0.00	16.20±0.00
FL	21.33±0.58^{a}	20.67±0.29^{ab}	20.33±0.29^{a}	20.67±0.73^{ab}	20.83±0.29^{ab}
LG	5.13±0.58^{b}	4.47±0.29^{ab}	4.13±0.29^{a}	4.47±0.76^{ab}	4.63±0.29^{ab}
SR	80.00	83.33	83.33	96.67	93.33

Means followed by different letters in the same rows are significantly different (P<0.05).
Please note that INW= initial mean weight, FMW= final mean weight, PER =protein efficiency ratio, CF=condition factor, FCR= feed conversion ratio, NM = nitrogen metabolism, WG =weight gain, SGR =specific growth rate, PWG =percentage weight gain, IL=initial length, FL= final length, LG= length gain, SR= survival rate

Table 6 : Haematology of Clarias gariepinus fed defatted G.mangostana- based diets at 0 and after 49 days

Parameters	Initial Value	Final values at Different DGM inclusion rates, %				
		Control	18gDGM	36g DGM	54g DGM	72gDGM
PCV (%)	26.00	32.00±1.41^{a}	32.00±1.41^{a}	30.00±2.83^{a}	30.00±1.41^{a}	34.00±1.41^{a}
Haemoglobin (g%)	8.00	10.00±1.41^{ab}	11.40±0.28^{b}	9.80±0.28^{ab}	9.6±0.14^{a}	11.30±0.14^{ab}
RBC x10^{12}/L	1.59	3.54±0.06^{b}	3.61±0.00^{b}	3.29±0.01^{a}	3.34±0.06^{a}	3.63±0.01^{b}
WBC x10^{9}/L	19425	19700±141.42^{d}	18100±141.42^{b}	25750±70.71^{e}	15900±70.71^{a}	18950±70.71^{c}
Platelet	107500	144000±5656.85^{c}	216000±2828.43^{d}	128000±1414.21^{a}	135900±212.13^{b}	128000±1414.21^{a}
Lymphocyte (%)	63.00	66.00±1.41^{a}	72.00±2.83^{b}	69.00±1.41^{ab}	71.00±1.41^{b}	71.00±1.41^{b}
Heterophil	34.00	29.00±1.41^{b}	22.00±2.83^{a}	23.00±1.41^{a}	19.00±1.41^{a}	22.00±2.83^{a}
Monocytes (%)	2.00	2.00±1.41^{a}	2.00±1.41^{a}	3.00±1.41^{a}	4.00±1.41^{a}	4.00±1.41^{a}
Eosinophils	1.00	3.00±1.41^{a}	4.00±1.41^{a}	5.00±1.41^{a}	6.00±1.41^{a}	3.00±0.00^{a}
Basophil	0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
MCHC (%)	30.00	31.38±5.81^{a}	31.58±0.79^{a}	32.77±2.15^{a}	32.03±1.04^{a}	33.15±0.82^{a}
MCH(Pg)	50.29	28.29±4.45^{a}	31.49±0.78^{a}	29.79±0.73^{a}	28.49±0.30^{a}	31.13±0.27^{a}
MCV(Fl)	163.28	90.38±2.56^{a}	94.18±3.92^{a}	91.17±8.19^{a}	86.06±0.36^{a}	93.66±3.53^{a}

*Means followed by different letters in the same rows are significantly different (p<0.05).*PCV=packed cell volume, RBC=Red blood cells, WBC=white blood cells, MCV = mean corpuscular volume, MCHC= mean corpuscular haemoglobin concentration, MCH = mean corpuscular haemoglobin

Table 7 : Blood biochemistry of Clarias gariepinus fed Defatted G.mangostana- based diets at 0 and 49 days

Parameter	Initial value	Final values at Different DGM inclusion rates				
		Control	18.00g DGM	36.00g DGM	54.00g DGM	72.00g DGM
Protein	3.3	3.90±0.14^{a}	3.50±0.14^{a}	3.90±0.14^{a}	3.80±0.14^{ab}	3.70±0.14^{ab}
Albumin	1.3	1.40±0.14^{a}	1.30±0.14^{a}	1.10±0.14^{a}	1.40±0.14^{a}	1.40±0.14^{a}

Globulin	2.0	2.50 ± 0.14^{ab}	2.20 ± 0.14^{a}	2.80 ± 0.14^{b}	2.40 ± 0.14^{a}	2.30 ± 0.14^{a}
AGR	0.65	0.60 ± 0.14^{a}	0.50 ± 0.14^{a}	0.30 ± 0.14^{a}	0.60 ± 0.14^{a}	0.60 ± 0.14^{a}
AST(IU/L)	35.5	33.00 ± 0.14^{a}	42.00 ± 0.14^{b}	36.00 ± 0.14^{a}	34.00 ± 0.14^{a}	40.00 ± 0.14^{b}
ALT(IU/IL)	20.5	26.00 ± 0.14^{b}	34.00 ± 0.14^{c}	29.00 ± 0.14^{b}	21.00 ± 0.14^{a}	32.00 ± 0.14^{c}

Means followed by different letters in the same rows are significantly different ($P<0.05$)

Table 8 : Tissue pathology of C. gariepinus post juveniles at 49 days after feeding on defatted G. mangostana-containing diets

Organs	Control	18g DGM	36g DGM	54g DGM	72gDGM
Gills	No visible lesions seen	No visible lesions seen	There is mild submucosal congestion	No visible lesions seen	No visible lesions seen
Kidney	No visible lesions seen	No visible lesions seen	No visible lesions seen	No visible lesions seen	There is severe interstitial congestion
Liver	No visible lesions seen	There are severe vacuolations of the hepatocytes, with mild central venous congestion	There is diffuse hepatic vacuolation.	No visible lesions seen.	There is diffuse vacuolation of the hepatocytes, marked
Heart	No visible lesions seen	No visible lesions seen	No visible lesions seen	No visible lesions seen	No visible lesions seen

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