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Ochang Stephen Ncha
Department of Fisheries and Aquatic Science, Cross River State University of Technology, Calabar, Nigeria.

Peter Bikom Michael
Department of Animal Science, Cross River State University of Technology, Calabar, Nigeria.

Ugbor Ogechi Nnabuchi
a) Fisheries and Hydrobiology Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria
b) Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

Egor Alex
Department of Fisheries and Aquatic Science, Cross River State University of Technology, Calabar, Nigeria.

Correspondence
Ochang Stephen Ncha
Department of Fisheries and Aquatic Science, Cross River State University of Technology, Calabar, Nigeria.

Effect of diets with moringa leaf meal on growth, carcass composition and haematology of *Clarias gariepinus*

Ochang Stephen Ncha, Peter Bikom Michael, Ugbor Ogechi Nnabuchi, Egor Alex

Abstract
A 56 - day feeding trial was carried out to study the effect of replacing the dietary soybean meal with *Moringa oleifera* leaves meal (MLM) on the growth performance, carcass composition and haematological indices of *Clarias gariepinus* fingerlings. Four iso-nitrogenous diets were formulated with MLM replacing 0, 20, 40 and 60% of SBM in the diets. The results showed significant difference ($p<0.05$) in weight gain, SGR, FCR and PER between treatments, with 20% MLM performing best. Significant difference ($p<0.05$) was recorded in carcass composition of fishes at the end of the trial. Protein content of the carcass reduced, and fat and ash contents increased as MLM increased in the diets. Though the haematological parameters were found to be significantly different ($p<0.05$) from the control diet, they were within normal range for *C. gariepinus.* The results of this study show that MLM can replace up to 20% of the soybean meal in the diets of *C. gariepinus*.

Keywords: *C. gariepinus*, *Moringa oleifera*, soybean, carcass and haematology

Introduction
Fish culture is growing very rapidly, with reported annual increase of about 10% [1]. Feed production needs to grow at the same rapid rate to meet the requirement of the sector. But the cost of fish meal is high and sustained production is not guaranteed because of dwindling catch from the wild. It becomes, therefore, necessary to evaluate other alternative protein sources for fish feed formulation. Protein of plant origin which are not conventionally used for human or livestock food or are not exploited for other industrial uses become attractive candidates for research.

Leaf meal comes under the category of non conventional plant protein feed ingredients. It is a source of vitamins, minerals and oxycarotenoids [2]. It has been reported that vegetable protein sources can supply fish with the protein needed for their maximum productivity [3]. One of such vegetable protein sources is *Moringa oleifera* leaves.

Moringa oleifera belongs to single generic family, Moringaceae and is found to be widely distributed in Africa and Asia. [4, 5] reported that *Moringa oleifera* leaf contains 86% DM, 29.7% CP, 4.38% CF, 29.9% EE, 3,056 kcal/kg energy, 0.26% calcium, with phosphorus and tannin (1.23g/kg) in negligible amounts. Moringa leaves have quality attributes that make them a potential replacement for soya bean meal or fish meal in non-ruminant diets. Moringa can be grown with ease, and has good coppicing ability, as well as good potential for forage production. The cost of producing large quantities of Moringa is low and does not require costly inputs to do well. [6] reported that moringa foliages can be harnessed as a cheap protein source for livestock feeding. One advantage of using moringa as a protein resource is that it is a perennial plant that can be harvested several times in one growing season. This has obvious economic advantage as lower feed cost will result in higher profit margin to the farmer. *Moringa oleifera* is in the group of high-yielding nutritious browse plants with every part having food value [7].

Recently, researchers have increasingly been paying attention to moringa (*Moringa oleifera* Lam.). Moringa leaf protein has been tested in the diets of many animals with variable success [8-11]. Few studies have been conducted using Moringa leaves meal on *Clarias gariepinus*, one of which is that of [12] that investigated the effect of moringa seed cake on *C. gariepinus.*

This present work is, thus, designed to evaluate the effect of replacing the soybean component of the diet of *C. gariepinus* with moringa leaves meal on the growth, haematology and carcass quality of the fish.

Materials and method

Source and processing of *Moringa oleifera* leaves

Moringa (*Moringa oleifera*) leaves used for this study were obtained from the Officers' Quarters of the Nigerian Prison Service, Obubra Local Government Area, Cross River State of Nigeria.

The leaves were thoroughly washed with water to remove dirt, drained properly and later shade dried for seven (7) days. Thereafter, the leaves were then milled into fine powder and analyzed for proximate composition.

Experimental diets

Four iso-nitrogenous (40% crude protein) experimental diets were formulated such that *Moringa oleifera* leaves meal (MLM) replaced soybean meal (SBM) at 20%, 40%, and 60% dietary levels respectively (Table 1). Diet with 0% *Moringa oleifera* leave meal served as control. Prior to processing, the feed ingredients were milled individually to a fine powder with the help of a hammer mill machine, then individually weighed and properly mixed together with adequate water added to ensure smooth pelleting. The feed pellets were sundried for three (3) days and the moisture content was found to be less than 10%. They were then bagged in airtight containers and stored until used.

Table 1: ingredient composition of the experimental diets (% dry matter)

	DIETS			
Ingredients	**D1**	**D2**	**D3**	**D4**
Blood meal (80% cp)	10.0	10.0	10.0	10.0
Fish meal (65% cp)	22.0	22.0	22.0	22.0
Soybean meal (44% cp)	38.0	30.4	22.8	15.2
Moringa leave meal (26% cp)	-	13.0	26.0	39.0
Maize (10% cp)	20.0	14.6	9.2	3.8
Vitamin /mineral premix	6.0	6.0	6.0	6.0
Binder	2.0	2.0	2.0	2.0
Total	100	100	100	100
Calculated crude protein levels	41.02	40.52	40.01	39.5

Experimental Design and Feeding Trials

This study was carried out in 12 (twelve) aquaria and with 240 fingerlings which were stocked at a density of 20 per aquarium and fed at 5% body weight for 8 weeks. The fish when brought to the laboratory was allowed to acclimate for 2 weeks. During the period of acclimation, the fish were fed commercial diets. The mean initial weight of the fish was taken with an electronic sensitive scale (Digital meter) and the initial mean total lengths measured with graduated rule and recorded. The 12 aquaria were randomly allocated to the four treatment diets (D1, D2, D3, D4) in triplicate and fish were randomly distributed into the aquaria at a stocking density of twenty fingerlings per aquarium. All fish were starved for 24 hours prior to introduction to experimental diets. This practice was done to eliminate variation in weight due to residue food in the gut and also to prepare the gastrointestinal tract for the experimental diets, while at the same time to increase the appetite of the fish. Feeding was carried out twice daily, (8.00-8.30 hrs) and (17.00 hrs-17.30 hrs). Subsequently, growth data were taken fortnightly and quantity of feed fed adjusted in accordance with the fish weight.

At the end of the experiment, all fish were weighed and data obtained from triplicate tanks were used to calculate weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR) and feed intake.

Weight gain = final weight – initial weight,

SGR = (LnW2 - LnW1)/ (T2-T1)100

Where W1 and W2 = initial and final weight of fish and T1 and T2 = time in days.

FCR = feed fed/live weight gain, PER = weight gain/protein in feed.

Carcass analysis

Three fish were chosen randomly at the beginning and at the end of the experiment randomly from each tank and subjected to chemical analysis of whole fish body.

Blood collection and haematological analysis

Fish were tranquilized with 150 mg/1 solution of tricane methanesulfonate (MS222) [13] for blood collection. Blood samples were collected from 3 fish at the beginning of the experiment and fortnightly, subsequently from each tank from the caudal artery using 2 ml plastic syringes and needle treated with anti-coagulant. Collected samples were put in sample bottles. Haematocrit (PCV) was determined with microhematocrit centrifuge by the Wintrobe and Westergreen method as described by [14] with commercially available heparinized capillary tubes of 25 mm. Red Blood Cell (RBC) and White Blood Cell (WBC) counts were determined with a haemocytometer with improved Neubauer counting chamber as described by [14]. Haemoglobin (Hb) concentration estimates were determined as described by [15]. The following parameters where calculated: mean corpuscular haemoglobin concentration (MCHC); mean corpuscular haemoglobin (MCH) and mean cell volume (MCV).

MCHC = Hb/PCV x 100;

MCV = PCV/RBC x 10;

MCH = Hb/RBC x 10.

Statistical analysis

All growth and hematological data were subjected to one way analysis of variance (ANOVA). Significant differences between means were determined by Duncan's Multiple Range test ($p<0.05$) using SPSS for windows (Version 20). Values were expressed as means ± SE.

Results

The proximate composition of the experimental diets and MLM are presented in Table 2. The crude protein content of the MLM used in this study is smaller than the values obtained in some studies in the literature. The result revealed that there was no significant ($p<0.05$) difference in the crude protein and moisture contents of the test diets. However, there exist a significant difference in the total fat, carbohydrate, crude fiber, ash, dry matter and energy among the diets. It could be observed from the table that while total fat and crude fiber decreased with increased incorporation of MLM, carbohydrate and dry matter content increased. Analysis of the anti-nutritional factors present in the test diets revealed the presence of oxalates, cynates, tryptic and phytates at varying levels (Table 3). From Table 3, a linear positive relationship exists between the level of anti-nutrients and the quantity of MLM incorporated in the diets.

Table 2: Proximate composition of the test diets and moringa leaves meal

Feed Samples	Total protein	Total fat	Total carbohydrate	Crude fibre	ash content	moisture content	dry matter	Energy
D1	42.00±2.00^{a}	9.43±0.55^{a}	43.00±1.00^{d}	2.03±0.15	9.50±0.50^{a}	9.00±0.20^{a}	86.66±0.58^{d}	1.79±1.00^{a}
D2	43.33±1.15^{a}	6.33±0.1n5^{b}	46.00±2.65^{c}	6.40±0.36^{a}	9.43±0.38^{a}	8.23±0.25^{a}	88.33±0.58^{c}	1.77±13.89^{b}
D3	43.33±0.58^{a}	5.43±0.21^{c}	48.67±0.58^{b}	5.27±0.32^{b}	8.57±0.32^{a}	8.63±0.13^{a}	89.66±0.58^{b}	1.76±11.00^{b}
D4	42.67±1.15^{a}	3.50±0.10^{d}	50.00±1.00^{a}	3.27±0.32^{c}	9.57±0.12^{a}	8.86±0.15^{a}	92.33±0.58^{a}	1.72±2.00^{c}
MLM	23.56±0.56	2.21±0.08	54.02±0.97	8.10±0.66	8.71±1.13	7.13±0.60	95.00±2.00	1.39±1.52

Means with the same superscripts are not significantly different at (P > 0.05)

The result obtained for the growth response, nutrient utilization and survival parameters of fish fed MLM based diet during the experiment are presented in Table 3. The fish fed D2 (20% MLM) diet gained 8.36±17 g, while the fish fed control diet (0% MLM) gained 8.11±3.06g. The values obtained for the fish fed control diet and 20% MLM diet were not significantly different (p>0.05) but were significantly different (p<0.05) when compared with fish fed 40% and 60% MLM based diets.

There was no significant difference (P>0.05) in the feed conversion ratio (FCR) in the fish fed control diet, 20%, and 40% MLM diet but there was a significant difference when compared with the fish fed the diet containing 60% MLM.

The results for the specific growth rate (SGR) showed that fish fed 0% MLM diet recorded the highest value of 6.59 and lowest value of 5.86 was recorded in fish fed 60% MLM diet. There was no significant difference (p>0.05) between fish fed 0% and 20%MLM diet as revealed in Table 3.

The highest value recorded for the highest protein efficiency ratio (PER) of 0.27 was observed in fish fed diet containing 0% MLM while the lowest value of 0.16 was recorded in fish fed diet containing 60%MLM.

Feed intake followed the same trend with PER. However, the result of the fish survival revealed that fish fed 60%MLM diet had the highest percentage survival while the fish fed 0% MLM had the least survival.

Table 3: growth responses and nutrient utilization of *C. gariepinus* fed MLM based diets for eight weeks

	DIETS			
Parameters	**D1**	**D2**	**D3**	**D4**
Initial mean weight (g)	1.82±9.53^{a}	1.88±23.12^{a}	1.99±15.27^{a}	1.90±16.50^{a}
Final mean weight (g)	9.92±3.64^{a}	10.24±5.61^{a}	8.70±2.63^{b}	8.59±7.61^{b}
Mean weight gain (g)	8.11±3.06^{a}	8.36±17^{a}	6.71±16^{b}	6.69±75^{b}
FCR	1.35±0.32^{b}	1.32±1.2^{b}	1.40±0.19^{b}	1.90±16^{a}
SGR	6.59±0.09^{a}	6.38±0.34^{a}	6.19±0.23^{b}	5.86±0.09^{bc}
PER	0.27±0.01^{a}	0.26±0.23^{a}	0.19±0.04^{b}	0.16±0.10^{b}
Feed intake (g)	2.99±1.73^{a}	2.71±3.21^{a}	2.47±6.08^{b}	2.35±5.51^{b}
Survival (%)	93.33^{b}	98.66^{a}	98.33^{a}	99.66^{a}

FCR = Food conversion ratio; SGR = Specific growth rate; PER = Protein efficiency ratio

Table 4: Haematological indices of *C. gariepinus* fed graded levels of MLM for eight weeks

	DIETS				
Parameters	**Initial**	**D1**	**D2**	**D3**	**D4**
PCV (%)	26.9±0.28^{bc}	28.49±0.41^{a}	29.21±0.28^{a}	27.1±0.8^{b}	25.8±0.9^{d}
WBC($10^{3}mm^{3}$)	6.20±0.93^{c}	6.98±0.01^{b}	7.5±0.8^{b}	7.9±0.5^{a}	8.1±0.6^{a}
RBC ($10^{6}mm^{3}$)	3.10±1.00^{bc}	3.6±0.4^{a}	3.3±0.6^{b}	2.8±1.00^{c}	2.5±0.5^{d}
Hb (g/100ml)	9.00±0.78^{c}	9.9±0.06^{a}	9.6±0.1^{b}	8.8±0.60^{d}	8.0±1.2^{d}
MCHC (%)	27.39±2.3^{e}	31.79±1.8^{d}	33.1±0.9^{c}	37.6±0.2^{b}	39.8±1.2^{a}
MCH (Pg)	28.1±1.50^{c}	27.6±0.8^{d}	29.8±1.20^{c}	35.6±0.1^{b}	41.6±1.8^{a}
MCV (fl)	99.68±2.00^{c}	91.8±0.2^{d}	101.3±1.2^{c}	116±0.8^{b}	128±1.60^{a}
PLT	59.3±0.50^{c}	61.1±1.20^{c}	64.2±1.00^{b}	63.6±0.30^{b}	66.7±0.6^{a}

PCV = Packed cell volume; WBC = White blood cell; Hb = haemoglobin concentration; MCHC = Mean corpuscular haemoglobin concentration; MCH = Mean corpuscular haemoglobin; MCV = Mean corpuscular volume; PLT = Platelet

The result of the haematological indices of the fishes fed with MLM-based diets is presented in Table 4. The PCV of fishes fed 20% MLM diet were not significantly different (p>0.05) from the fish that were fed with the control diet. The result showed that fishes fed the control and 20% MLM diet had increase in the PCV. D3 (40% MLM) and D4 (60% MLM) exhibited a negative impact on the fishes.

The results for the WBC revealed a positive correlation between MLM and WBC of the fishes. The fishes fed D1 (control diet) and D2 (20% MLM diet) recorded values of 6.98 x $10^{3}mm^{-3}$ and 7.2 x $10^{3}mm^{-3}$ respectively. These values showed significant difference (p<0.05) from the values obtained in fishes fed diet containing 40% and 60% MLM (Table 4).

The result for RBC showed a decrease with increase in MLM in the test diets. D1 (0% MLM) recorded the highest value of 3.60 x $10^{6}mm^{-3}$ and did not differ significant (p>0.05) from the value of 3.30 x $10^{6}mm^{-3}$ obtained in fish fed D2 containing 20% MLM. Fishes fed diet containing 40% and 50%MLM diets showed decrease in RBC.

The result of the Hb showed that fishes fed D1 had significantly (p<0.05) higher values of Hb than the rest of the fishes fed D2 to D4. It was observed that Hb decreased with increase incorporation of MLM in the test diets.

MCHC, MCH and MCV increased with an increase in MLM in the diet, with D4 having the highest values.

Table 5: Carcass composition of *C. gariepinus* fed MLM based diets for eight weeks

Parameters						
Diets	**Carbohydrate**	**Protein**	**Fat**	**Ash**	**Fibre**	**Moisture**
D1	2.53±0.30[a]	21.00±5.56[a]	1.86±0.58[c]	2.06±0.20[b]	0.32±0.09[a]	73.56±4.21[a]
D2	2.46±0.21[a]	19.66±4.04[b]	2.17±0.39[c]	2.86±0.23[b]	0.36±0.12[a]	73.63±2.67[a]
D3	2.33±0.10[a]	19.00±1.00[b]	2.90±0.17[b]	3.05±0.15[a]	0.38±0.06[a]	75.86±1.25[a]
D4	2.37±0.04[a]	17.66±2.51[c]	3.15±0.34[a]	3.35±0.13[a]	0.38±0.08[a]	73.93±1.69[a]

Means with the same superscripts are not significantly different at (P > 0.05)

The result of the carcass composition of the fish fed MLM based diets is presented in Table 5. The result revealed that incorporation of MLM in the diets affected significantly ($p<0.05$) the protein, fat and ash content of the carcass of the fish. Whereas, the protein content of the fish carcass decreased with increased incorporation of MLM in the diets, fat content of the fish carcass decreased.

Discussion

The crude protein content of the MLM used in this research is less than 27.51% recorded by [16], and 29.68% reported by [10]. The variation in the nutrient content of the leaves could be attributed to the age of cutting, harvesting, climatic conditions, edaphic factors as well as methods of processing and analysis [17].

The growth and nutrient utilization by fish decreased as MLM increased in the diets. This finding is in agreement with the discoveries of previous studies. It has been reported that higher substitution of MLM with fish meal had a negative effect on the growth performance because of the presence of antinutrients such as phenol, tannins, phytates and saponins [18, 19]. Another plausible explanation for the observed decrease in growth and nutrient utilization maybe due to imbalanced protein to energy ratio in the test diets [19] as shown in Table 2.

Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish [20]. Haematological characteristics of most fish have been studied with the aim of establishing normal value range and deviation from it may indicate a disturbance in the physiological process [21]. Environmental and physiological factors are known to influence fish haematology; these include stress due to capturing, transportation, sampling, age and sex.

Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood [22]. All the haematological parameters measured in this study were within the recommended physiological ranges reported for *C. gariepinus.*

The PCV, RBC and Hb were observed to decrease as the level of MLM increased in the diet. Reduction in the concentration of the PCV in the blood usually suggests the presence of toxic factor which has adverse effect on blood formation [23]. The decrease in RBC may be ascribed to the higher concentration of anti-metabolites especially phytates in the diets containing more MLM [24]. The reduction in the Hb concentration could imply that diets having higher substitutions contained low quality protein, and this may have resulted in poor transportation of oxygen from the respiratory organs to the peripheral tissue [24].

White blood cells (WBC) are the defense cells of the body. It has been demonstrated that the quantity of WBC has implication in immune responses and the ability of the animal to fight infection [25]. WBC count showed an increase as the level of MLM increased in the diet. High WBC count is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system [23]. The increase in WBC as MLM increased in the diet could imply some form of feed toxicity

References

1. Bello NO, Nzeh GC. Effect of varying levels of *Moringa oleifera* leaf meal diet on growth performance, haematological indices and biochemical enzymes of African catfish, *Clarias gariepinus* (Burchell, 1822). Elixir Aquaculture 2013; 57A:14459-14466.
2. Opara CC. Studies on the use of *Alchornea cordifolia* leaf meal as feed ingredients in poultry diets. International Journal of Poultry Science. 1996; 9(4):363-367.
3. Nwanna LC, Falaye AE, Sotolu AO. Water hyacinth *Eichhomia crassipes* (Mart solms): a sustainable protein source for fish feed in Nigeria. Food health and environmental issues in developing countries: the Nigeria situation. Adeboye OC, Taiwo KA, Fatufe AA (Editors). Alexander Von Humboldt Foundation, Bonn - Germany, 2008, 187-194.
4. Francis G, Makkar HPS, Beckar K. Plant toxins and detoxification methods to improve and extract *Moringa oleifera* leaves. Pakistan Journal of Nutrition. 1999; 7(1)13-16.
5. Kakengi AMV, Shem MN, Sarwatt SV, Fujihara T. Can *Moringa oleifera* be used as a protein supplement to ruminants? Asian-Australasian Journal of Animal Sciences. 2003; 18(1):42-47.
6. Sarwatt S, Milang'ha M, Lekule F, Madala N. *Moringa oleifera* and cottonseed cake as supplements for smallholders' dairy cows fed Napier grass. Livestock Research for Rural Development 2004; 16:6-13.
7. Duke AJ. Moringaceae. Handbook of Energy Crops. Mc Graw Hill, New York, 1998.
8. Manh L, Nguyen N, Ngoi T. Introduction and evaluation of *Moringa oleifera* for biomass production and feed for goats in the Mekong delta. Livestock Research for Rural Development 2005; 17:9.
9. Reyes-Sánchez N, Spörndly E, Ledin I. Effect of feeding different levels of foliage of *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition. Livestock Science 2006; 1001:24-31.
10. Odetola OM, Adetola OO, Ijadunola TI, Adedeji OY, Adu OA. Utilisation of moringa (*Moringa oleifera*) leave meal as a replacement for soybean meal in rabbit's diets. Scholarly Journal of Agricultural Science. 2012; 2(2):309-313.
11. Aderinola OA, Rafui TA, Akinwumi AO, Alabi TA, Adeagbo OA. Utilisation of *Moringa oleifera* leaf as feed supplement in broiler diet. International Journal of Food, Agriculture and Veterinary Science. 2013; 3(3):94-102.
12. Bundit Y, Rungkan, K, Siripavie C. Partial replacement of protein in soybean meal by moringa seed cake (*Moringa oleifera*) in bocurtis catfish (*Pangasius bocourti*). Songklana-Karin Journal of Science and Toxicology. 2014; 36(2):125-135.
13. Wagner EJ, Jensen T, Arndt R, Routledge, MD, Brodwich Q. Effects of rearing density upon cutthroat hematology, hatchery performance, fin erosion and general health and condition. The Progressive Fish-Culturist 1997; 59:173-187.
14. Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. Journal of Fish Biology. 1973; 5:771-781.
15. Wedemeyer GA, Yastuke WT. Clinical methods for the assessment of the effects of environmental stress on fish health. United States Fish and Wildlife Service Technical papers, 1977, 89.
16. Oduro I, Elis WO, Owusu D. Nutritional potential of two leafy vegetable: *Moringa oleifera* and *Ipomea batatas* leaves. Scientific Research and Essay 2008; 3(2):57-60.
17. Fuglie L. Producing food without pesticides: local solution to crop pest control in West Africa, CJS, Wageningen, The Netherlands, 1999.
18. Richter N, Siddhuraju A, Becker K. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as alternative protein source for Tilapia (*Oreochromis niloticus* L) Aquaculture 2003; 217:599-611.
19. Eyo JE. Effect of substituting soybean meal for maggot meal on acceptability of diet, growth performance and cost benefit of diet to hybrid catfish. Journal of Agricultural Research. 2005; 10:20-25.

20. Hrubec TC, Cardinale JL, Smith SA. Haematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid). Veterinary Clinical Pathology 2000; 29:7-12.
21. Rainza-paiva MJT, Ishikawa CM, Das-Eiras AA, Felizardo NN. Haematological analysis of chara *Pseudoplatystoma fasciatum* in captivity. Aqua Responsible aquaculture in the new millennium. Nice, France. May 2-6 2000. European Aquaculture Society, Special Publication 2000; 28:590-561.
22. Oyawoye EO, Ogunkunle M. Chemical analysis and biochemical effects of raw Jack beans on broiler. Proceedings of Nigeria Society of Animal Production 1998; 23:141-142.
23. Osuigwe DI, Obiekezie AI, Onuoha GC. Some haematorlogical changes in hybrid catfish, *Heterobranchus longifilis* x *Clarias gariepinus* fed different dietary level of raw and boiled jackbean, *Canavalia ensiforms* seed meal. African Journal of Biotechnology. 2005; 4(9):1017-1021.
24. Roberts KM, Daryl KG, Peter AM, Victor WR. Mayer's Biochemistry, 25th edition, Mc Graw Hill, New York, 2000.
25. Douglas JW, Janes KW. Schalm's Veterinary Haematology. John Wiley and Sons. Blackwell Publishing Ltd, New York, 2010.