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Proximate And Phyto-Chemical Contents Of Selected Leave Meals As Alternative Feed Ingredients For Fish Feed Production

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ABSTRACT

The present study evaluates the nutritional potential of four different plant leaves as alternative to conventional feed ingredients for fish feed production. These leaves include; moringa leaf meal, leucaena leaf meal, sweet potato leaf meal, groundnut leaf meal, and cassava leaf. The crude protein content of the ingredients ranged from 21.88 to 36.05% and the crude lipid contents were recorded as 2.13 to 3.84% with moringa leave meal having the highest value. All the tested ingredients contained phytic acid (10.69 to 16.49 mg/100g), oxalate (494.00 to 879.50 mg/100g) tannin (52.78 to 136.93 mg/100g) and cyanide (4.36 to 10.53 mg/100g) with moringa also having higher content of these antinutrients. It however expected that with appropriate processing methods this ingredients can be made more suitable for incorporation in fish feed.

INTRODUCTION

Fish feed play major role in aquaculture viability and profitability, because it accounts for at least 40 - 60% of the total cost of fish production (Jamu and Ayinla, 2003). Although there are rooms for enhancing aquaculture production in Africa through improvements in the overall production system, in genetics and general farm management principles, the desired growth of aquaculture which is necessary in order to meet the increasing demand for fish is only achievable through cost-effective and high quality fish feed (Gabriel et al, 2007).

Locally produced feed reduces the cost of production and hence, cheaper means of meeting the protein requirement improve food security and reducing the level of poverty in developing countries, thus inexpensive and locally available feedstuffs are to be identified. The search for alternative protein sources has focused on by-products and materials which are not suitable for direct human consumption (Hoffman et al, 1997).

It is also advantageous if the target feed stuffs are locally available and in abundance, affordable, and does not conflict with human food security interests and are not used for animal feed industry. One of such feed stuff are plant leave meal. To develop low cost and quality fish feed, nutritional information about proximate composition and phytochemical composition of the locally available feed ingredients is very essential. Therefore, the present study was conducted to evaluate the nutritional and anti-nutritional contents of selected locally available plant based ingredients to find their suitability to be used as fish feed ingredients.

MATERIALS AND METHODS

The leaves of the five selected plants (*Moringa oleifera, Leuceana leucocephala, Ipomea batata, Manihot esculenta, and Arachis hypogea*) were collected from University of Agriculture Research Farms, Benue State, Nigeria. The leaves were washed and air dried. After drying, the leaves were ground into a fine powder using laboratory mortar and pestle, and then sieved and stored in a well labelled air-tight container for analysis.

Experimental site

Proximate composition and Anti-nutritional factors of the leaves (*Moringa oleifera, Leuceana leucocephala, Ipomea batata, Manihot esculenta, and Arachis hypogea*) were conducted at the University of Jos, Jos, Nigeria.

Analytical procedure

The proximate analysis for the various constituents was carried out based on the description of the Association of Official Analytical Chemist (A.O.A.C, 2000). Moisture content determination involved washing a known weight of sample with clean and distilled water and drying to a constant weight at 60oC in an oven (Gallen Kamp hot box). Determination of ash involved incineration in a muffle furnace (Gallenkamp hot box) at 550oC for 24 hours or a dried, ground sample was ignited in a furnace at 550oC to oxidize all organic matter. Ash was determined by weighing the resulting inorganic residue.

Crude fat determination involved using exhaustive soxhlet extraction of a known weight of sample with petroleum ether (b.pt 40-60oC) and methanol mixed properly in the ratio 1:1. Crude fibre was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat-free samples with 1.25% each of the sulphuric acid and sodium hydroxide solutions under specified condition.

$$\% \text{ Fibre} = \frac{\text{loss of weight on ignition}}{\text{weight of sample used}} \times 100$$

Determination of crude protein was done using the microkjeldahl nitrogen method which involves the digestion of a given weight of the sample with concentrated H_2SO_4 and a catalyst to convert any organic nitrogen to ammonium sulphate in solution, followed by the decomposition of ammonium sulphate with NaOH. The ammonia liberated was distilled into 5% boric acid. The nitrogen from ammonia was deduced from titration of the trapped ammonia with O.O5N HCl using methylene red and methylene blue (double indicator solution) indicators. The value of nitrogen obtained was multiplied by 6.25 to give the % crude protein.

Determination of anti-nutritional factors

Anti- nutrient contents was determined by the following methods:

Total oxalate: total oxalate was determined by the permanganate titration method of Dye (1956).

Phytic acid: Dried sample was soaked in 2% HCl solution and filtered. The solution was titrated against a standard iron III Chloride solution containing 0.00195 g of iron per milliliter in the presence of 0.3% ammonium thiocyanate. The percentage phytic acid was calculated (Lucas and Markakas 1975).

$$\% \text{ Phytic acid} = \gamma \times 1.19 \times 100$$
$$\gamma = \text{Titre value} \times 0.00195$$

Cyanide content: Powdered sample was soaked in a mixture containing distilled water and orthophosphoric acid for 24 hr. The resulting sample was distilled and the distillate was titrated against 0.02 N $AgNO_3$ in the presence of 5% potassium iodide (1 mL of 0.02 N $AgNO_3$ = 1.08 mg HCN) (AOAC, 1990).

Tannin content: Powdered sample was extracted into 70% acetone. Sample extract aliquot was mixed with folincioalteau reagent, 10% Na2CO3 and incubated for 40 minutes at room temperature. The absorbance was measured at 700 nm. Tannin content was expressed as mg/ 100 g Tannic acid equivalents (Makkar et al. 1993).

RESULTS AND DISCUSSION

Proximate composition of the selected plant ingredients

Data on proximate composition of the selected leaves are presented in table (i). The crude protein contents were in the range of 21.88% ± 0.66 to 36.05% ± 0.60. Within this group of feedstuffs, moringa (Moringa oleifera) leaf meal had the highest crude protein content 36.05% while cassava (Manihot esculenta) leaf meal had the lowest crude protein content of 21.88%. There was no significant difference (p>0.05) in CP between leucaena (Leucaena leucocephala) leaf meal and sweet potato (Ipomea batata) leaf meal which had 33.77% CP and 33.35% CP respectively. Leucaena leaf meal had the highest moisture content of 6.30% ± 0.70 while the lowest moisture content of 4.71%±0.14 was recorded for cassava (Manihot esculenta) leaf meal. There was no statistical variation (P>0.05) in moisture value between leucaena leaf meal and groundnut (Arachis hypogaea) leaf meal and between Moringna leaf meal and sweet potato leaf meal respectively, while moisture content of cassava leaf meal (4.71 ± 0.14) differ significantly (P<0.05) for all the other leaf meals. Ether extract recorded range from 3.84%± 0.08 to 2.13% ± 0.17. Moringa leaf meal recorded the highest Ether extract of (3.84 ± 0.08) which was significantly different (P<0.05) from all other leaf meals while sweet potato leaf meal had the lowest with 2.13% ± 0.17. However, there was no statistical significant difference (P>0.05) in EE between cassava leaf meal, leucaena leaf meal and groundnut leaf meal. The highest crude fibre was recorded in cassava leaf meal with CF of 11.08% ± 0.15 followed by sweet potato leaf meal with CF of 10.54 ± 0.25 which were not significantly different (P>0.05). The lowest CF of 5.33% ± 0.23 was recorded for groundnut leaf meal. There was no significant variation (P>0.05) in CF value of 7.92% ± 0.28 and 8.53 ± 0.26 recorded for leucaena and moringa leaf meal respectively. Moringa leaf meal had the highest Ash content of 12.95% ± 0.08 followed by sweet potato leaf meal with Ash content of 9.27% ± 0.17. However, there was no significant difference (P>0.05) in Ash values of 8.17% ± 0.17, 8.09% ± 0.04 and 8.44% ± 0.18 which were recorded for leucaena, sweet potato and cassava leaf meals respectively. Groundnut leaf meal recorded the highest Nitrogen free Extract (NFE) of 61.42 ± 0.98 and the lowest NFE of 38.65± 0.3 recorded for moringa leaf meal. However, NFE values of 47.29 ± 0.14, 49.50 ± 0.17 and 50.77 ± 0.47 recorded for leucaena, cassava and sweet potato leaf meals respectively did not show any statistical significant difference (p>0.05).

Anti-nutritional composition of the selected plant ingredients

Table (ii) shows the anti-nutrient composition in mg/g of the five selected leaf meals. The values for the oxalate varied from 494.00mg/100g ± 1.00 to 879.50mg/100g ± 0.50. The highest value of 879.50mg/100g ± 0.50 was recorded in moringa with the lowest value of 494.00mg/100g ± 01.00 recorded for cassava leaf meal. The values, 791.00mg/100g ± 1.00, 692.50mg/100g, and 571.50mg/100g were recorded for groundnut, leucaena and sweet potato leaf meals respectively. The result obtained for tannin in the leaf meals were in the range of 52.78mg/100g ± 0.23 to 136.93mg/100g ± 0.25, with the highest value of 136.93mg/100g ± 0.25 obtained for sweet potato leaf meal and the lowest value (52.78mg/100g ± 0.23) obtained in cassava leaf meal. The highest content (16.49mg/100g ± 0.18) of phytic acid was found in moringa leaf meal with lowest value 0f 10.69mg/100g ± 0.19 was recorded for sweet potato leaf meal. Values obtained for cyanide were in the range of 4.36mg/100g ± 0.15 to 10.53mg/100g ± 0.18. The values of 9.56mg/100g ± 0.20, 7.19mg/100g ± 0.25 and 6.13mg/100g ± 0.23 were recorded for leucaena, groundnut and sweet potato leaf meals respectively.

Table 1 : Proximate composition of the plant based ingredients

Parameters (%)	Leuceana leaves	Groundnut leaves	Moringa leaves	Cassava leaves	Sweet potato leaves	P-Value
Crude Protein	33.77 ± 0.42^{b}	21.88 ± 0.66^{d}	36.05 ± 0.60^{a}	27.96 ± 0.24^{c}	33.35 ± 0.52^{b}	0.001
Moisture	6.30 ± 0.70^{a}	6.54 ± 0.05^{a}	5.63 ± 0.12^{b}	4.71 ± 0.14^{c}	5.98 ± 0.04^{b}	0.002
Crude fat	2.87 ± 0.17^{c}	3.28 ± 0.08^{b}	3.84 ± 0.08^{a}	3.03 ± 0.03^{bc}	2.13 ± 0.17^{d}	0.003
Crude Fibre	7.92 ± 0.28^{b}	5.33 ± 0.23^{c}	8.53 ± 0.26^{b}	11.08 ± 0.15^{a}	10.54 ± 0.25^{a}	0.003
Ash	8.17 ± 0.17^{c}	8.09 ± 0.14^{c}	12.95 ± 0.08^{a}	8.44 ± 0.18^{c}	9.27 ± 0.17^{b}	0.007
NFE	47.29 ± 0.14^{b}	61.42 ± 0.98^{a}	38.65 ± 0.34^{c}	49.50 ± 0.17^{b}	50.77 ± 5.47^{b}	0.002

Mean in the same row with different superscripts differ significantly (P<0.05)

Table 2: Level of anti- nutrient content in the plant based ingredients

Parameters (mg/100g)	Leuceana leaves	Groundnut leaves	Moringa leaves	Cassava leaves	Sweet potato leaves	P-Value
Oxalate	692.50 ± 0.50^{c}	791.00 ± 1.00^{b}	879.50 ± 0.50^{a}	494.00 ± 1.00^{e}	571.50 ± 0.50^{d}	0.001
Tannin	83.79 ± 0.38^{c}	89.87 ± 0.54^{b}	62.23 ± 0.12^{d}	52.78 ± 0.23^{e}	136.93 ± 0.25^{a}	0.002
Phytic acid	11.78 ± 0.28^{b}	12.31 ± 0.14^{b}	16.49 ± 0.18^{a}	11.92 ± 0.09^{c}	10.69 ± 0.19^{d}	0.003
Cyanide	9.56 ± 0.20^{b}	7.19 ± 0.25^{c}	10.53 ± 0.18^{a}	4.36 ± 0.15^{e}	6.13 ± 0.23^{d}	0.003

Mean in the same row with different superscripts differ significantly (P<0.05)

DISCUSSION

The potentials of feedstuffs such as leaf meal in fish diets can be evaluated on the basis of its proximate chemical composition, which comprises the moisture content, crude protein, crude fibre, crude lipid, total ash and Nitrogen free extract (Adewolu, 2008). Protein is the major growth promoting factor in feed (Mahmud et al; 2012). The protein contents of the ingredients used in the present study were in the range of 21.88% to 36.05%, but comparatively lower when compared with fish meal which contains 50 to 70% protein content (FAO 2013). All the five ingredients used for this present study meet the National Research Council (NRC) standard or specification on nutrient requirement of fish for protein feed classification of ≥ 20 CP. Any feed stuff that is ≥ 20 CP can be referred to as protein supplement (NRC 1993). The Proximate compositions of M. *oleifera* leaf meal (36.05%) and L. *leucocephala* (33.77%) in the present investigation fall within the range obtained by Madalla et al (2013) and Adeparusi and Agbede (2010). The protein content of sweet potato leaves meal (33.35%) was higher when compared with values (28.85% and 23.35%) recorded by Antia et al 2006 and Adewolu (2008). In the present study, cassava leaf meal recorded 27.96% CP. Iheukwumere et al (2008) reported 25.10% while Ekanem et al (2010) reported 25% crude protein for cassava leaf meal both which conform to the present study. However, the crude protein for cassava leaf meal (27.96%) and groundnut leaf meal (21.88%) under the present investigation fall below the values (33.2% and 27.8%) reported by Fasuyi (2005). These differences might be due to different environmental conditions such as soil type, harvesting time, local varieties and processing methods. Lipid content of the ingredients is an important factor in fish feed formulation as lipid supply essential fatty acids (EFA) and serves transporters for fat-soluble Vitamins (Graig and Helfrich, 2009). Crude lipid content recorded in this study was ranged from 2.13 to 3.84g/100g with the highest lipid content (3.84g/100g) recorded in M. *oleifera* leaf meal. These observation is in close range with the values (1.64 to 3.94g/100g) reported by Paranamana et al 2015 of lipid contents of some plant leaves. Fibre plays a nutritional role in moving the bolus and absorbing toxins (Paranamana et al, 2015). In fact, the amount of crude fibre in fish feeds is usually less than 7% of the diet to limit the amount of undigested material entering the culture system (Delbert, 2010). Except for ground nut leaf meal (5.33g/100g) all the other ingredients used in the present study exceed the limit. Highest amount of Ash content was from M. oleifera leaf meal (12.95g/100g) and Ash contents in the other ingredients were ranged from 8.09 to12.95%. High Ash content reduces the digestibility of ingredients in the diet resulting in poor growth of fish and Ash is not desirable to exceed 12% (De Silva and Anderson, 1995). The values of Ash in the present study fall within this limit (12%) for diet of fish.

Nutritive value of plant ingredients influenced the growth of fish which is constrained by anti-nutritional factors (Paranamana et al, 2015). Although all the plant derived materials analysed in the present study have potential to be used as fish feed ingredients. The presence of anti-nutrients causes to reduce the nutritional quality of the fish feeds (Francis et al; 2001). All the plant materials in the present study showed the presence of anti-nutrients such as Phytic acid, cyanide, oxalate and

and tannin. The values of Cyanide recorded in this study showed lower contents to values reported by paranamana et al (2015) while Phytic acds and tannin level tested recorded a much higher values than the works of Francis et al (2001) and paranamana et al (2015) in the leaf meals of different plants ingredients such as bambara groundnut leaves, sweet potato leaves, pawpaw leaves, banana leaves, and soybeans leaves. Phytic acid level in fish feeds above the concentration of 0.5% was detrimental to the growth of fish (Francis et al, 2001). The phytic acid levels in the present study which varied from 10.69mg/100g to 16.49mg/100g exceed this level of 0.5%. It has been shown that tannin interfere with the digestive processes by inhibiting protease and also forming indigestible complexes with dietary protein at inclusion rate of 2g/100g (Berker and Marker, 1999). The levels (52.78mg/100g to 136.93mg/100g) recorded for tannin in all the analyzed ingredients in the present study indicated a lower value than this inclusion rate of 2g/100g. No exact limit of toxicity of cyanide for fish has been reported yet and more studies are required to determine the cyanide level of tolerance for fish (Francis et al, 2001) but Davies (1991) reported that cyanides derived from hydrolysis of cyanogens can suppress natural respiration and cause cardiac arrest. All the ingredients contained 10.23mg/100g to 30.02mg/100g of cyanide in the present study. Oxalates affects calcium and magnesium metabolism and react with proteins to form complexes which have an inhibitory effect in peptic digestion (McDonald et al., 1995). Oxalate level up to 10 to 20ppm/kg is within tolerable limit for man but the tolerable range for fish has not been reported (Balogun, 2013). The result shows an exceptional high value (494mg/100g to 879.50mg/100g) of oxalate when compared to 308mg/100g in sweet potato leaves (*Ipomea batata*), and 95.50mg/100g in bitter leaves (*Vernonia amygalina*) reported by Antia et al. (2006). In general, the values of pytic acids, tannins and cyanide recorded in the present study showed lower values than the value recorded by Paranamana et al (2015) in the study of plants leaf ingredients in fish feed while Oxalate values correspond to the work of Francis et al; 2001 and Paranamana et al (2015).

Conclusively, the results reveals that the investigated leaves contains an appreciable amount of proteins, fat and other vital constituent and low levels of toxicant except for Oxalate whose value can be reduced by employing a suitable processing method. As a result, all the above investigated leaves have the potential to serve as fish feed ingredients and therefore, recommend inclusion of these plant feedstuffs in fish diets in order to minimize the cost of fish production among small scale fish farmers. However, before wholesale utilization of these plant feedstuffs in fish diets, there is also need for further research to evaluate among others, the amino acid profile, digestibility and processing methods of these plant feedstuffs.

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Disclosure statement

No potential conflict of interest was reported by the author.

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REFERENCES

1.O. A. C.(2000); Association of Official Analytical Chemists International (AOAC). Official Methods of Analysis, 17th ed. AOAC International, Gaithersburg, MD, USA.
2. Adeparus E. O and Agbede J. O. (2010); evaluation of Leucaena and Gliricidia leaf protein concentrate as supplements to bambara groundnut (Vignas subterranean(l.verdc) in the diet of oreochromis niloticus. Fisheries and Wildlife Department, Federal University of Technology, Akure, Ondo State, Nigeria.
3. Adewolu M .A. (2008); Potentials of Sweet Potato (Ipomoea batatas) Leaf Meal as Dietary Ingredient for Tilapia zilli Fingerlings. *Pakistan Journal of Nutrition* 7 (3): 444-449, 2008.
4. Antia B. S, Akpan E. J, Okon P.A and Umoren I. U. (2006); Nutritive and Anti-Nutritive Evaluation of Sweet Potatoes (Ipomoea batatas) Leaves. *Pakistan Journal of Nutrition* 5 (2): 166-168.
5.AOAC (1990); Official Methods of Analysis. 15th edn. Association of Official Analytical Chemists. Washington, DC.
6. Balogun, B.I. (2013); Effects of Processing Methods on Anti-Nutrient Levels of Bauhinia monandraKurz. Seed. *Academic journal of Interdisciplinary Studies* ; 9 (2): 88-101.
7. Becker K and Makkar H P S. (1999); Effects of dietary tannic acid and quebracho tannin on growth performance and metabolic rates of common carp (*Cyprinus carpio L.*). *Aquaculture* 175: 327–35.
8.Bello, Nuhu Ozovehe and G.C. Nzeh (2013); Effects of varying levels of Moringa oleifera leaf meal diet on growth performance, hematological indices and biochemical enzymes of African catfish Clarias gariepinus (Burchell 1822). *Elixir Aquaculture 57A (2013) 14459-14466*
9. Craig S. and Helfrich L. (2009); Understanding Fish Nutrition, Feeds, and Feeding. Cooperative Extension Service. Publication 420–256.http://pubs.ext.vt.edu/420256/.
10. Davies R. H. (1991); Cyanogens. D'Mello F P J, Duffus C M, Duffus J H.(Eds.), Toxic Substances in Crop Plants. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge CB44WF, Cambridge, pp. 202–25.
11. De Silva S S and Anderson T A. (1995); Fish Nutrition in Aquaculture. 1st edn. Chapman and Hall, 2–6, Boundry Row, London, SE 1 8HN, UK.
12. Delbert M. Gatlin III (2010); Principles of Fish Nutrition. Southern Regional Aquaculture Center. SRAC Publication No. 5003.
13. Dye, W.B. (1956); Studies on Halogeton *glomerulus*. *Weeds* 4: 55-59.
14. Ekanem AP, Eyo VO, Ndome CB (2010); The Effect of diet with different inclusion level of Cassava leaf meal (CLM) Mahinot utilization on the growth performance of *Heteroclarias fingerings*. J*ournal of Sciences and Multidisciplinary Research* 2: 58- 67.
15. El-saidy, D. M. S. and M. M. A. GABER (2004); Use of cottonseed meal supplemented with iron for detoxication of gossypol as a total replacement of fishmeal in Nile *tilapia*, *Oreochromis niloticus* (L.) diets. *Aquacult. Res*. 34 (13), 1119–1127.
16. Food and Agriculture Organization of the United Nations; (2013); Aquaculture Feed and Fertilizer Resource Atlas of the Philippines. Food and Agriculture organization of the United Nations. FAO publishers.
17. 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.
18. Gabriel U. U, Akinrotimi OA, Bekibele DO, Onunkwo DN, Anyawu PE (2007); Locally fish feed, potentials for aquaculture development in Sub–Saharan. *Afr J Agric Res*, 2(7):287–295.
19. Hoffman L C Prinson J F and Rukan G (1997); Partial replacement of fish meal with either soybean meal or brewery yeast or tomato meal in the diet of African sharp tooth Catfish Clarias gariepinus. Water SA 23(2), 181 – 186.
20. Hossain, M. A., U. Focken and K. Becker (2002); Nutritional evaluation of dhaincha (Sesbania aculeate) seeds as dietary protein source for tilapia Oreochromis niloticus. *Aquacult. Res*. 33, 653 662.
21. Iheukwumere FC, Ndubuisi EC, Mazi EA, Onyekwere MU (2008); Performance, nutrient utilization and organ characteristics of broilers fed cassava leaf meal (Manihotesculenta Crantz). *Pakistan Journal of Nutrition* 7:13-16.
22. Jamu DM and Ayinla OA (2003); Potential for the development of aquaculture in Africa. *African Journal of Agricultural Research.* 2(7), pp. 287-295
23. Lucas G M and Markakas P. (1975); Phytic acid and other phosphorous compounds of bean (Phaseolus vulgaris). *Journal of Agriculture Education Chemistry* 23: 13–15.
24. Madalla N, Agbo N.W and Jauncey K (2013); Evaluation of Aqueous Extracted Moringa Leaf Meal as a Protein Source for Nile Tilapia Juveniles. *Tanzania Journal of Agricultural Sciences* (2013) Vol. 12 No. 1, 53-64.
25. Mahamud N A, Minar M H, Hasan M D R and Hossain M B (2012); Proximate composition of fish feed ingredients available in Lakshmipur region, Bangladesh. *American-Eurasian Journal of Agriculture & Environmental Science* ,12 (5): 556– 60.
26. Makkar H P S, Blümmel M, Borowy N K and Becker K (1993); Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of Science Food Agriculture* ,61: 161–65.
27. McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan (1995); Animal nutrition. 5th Edn., Longman group Ltd., UK., pp: 607.
28. NRC (1993); National Research Council. Nutrient Requirement of Fish. National Academy Press, Washington, DC, pp: 114.
29. Oberlease, D (1973); phytates In: Toxicants occurring naturally in Foods (Strong, F. ed) National Academic of sciences, Washington D.C. pp 363-144.
30. Paranamana N., Radampola K. And Bulugahapitiya V. P. (2015); Nutritional and nti nutritional contents of alternative plant feed ingredients for fish feed formulation. I*ndian Journal of Animal Sciences* 85 (2): 212–215.
31. Rinchard J, Mbainzireki G, Dabrowski K, Lee K J, Garcia-Abiabo M. A and Ottobre J. (2002); The effects of cottonseed meal protein level on growth, gonad development and plasma sex steroid hormones of Tropical fish Tilapia, Oreochromis sp. *Aquaculture International* 10, 11-28.