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Research Paper

The use of tadpole meal as a substitute for fish meal in diets of *clarias gariepinus* fingerlings

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Two hundred and forty *Bufo maculate* tadpoles of weight ranging from 0.038-0.045g (mean weight 0.04±0.008)g and lengths from 1.2-1.6 cm (mean 1.4± 0.018 cm) were randomly selected from breeding tanks (2.0H 2.0H1.0 m) and raised in utilization outdoor concrete tanks (1.0H1.0H0.75 m) for 84 days at the front of faculty of science complex, University of Abuja, Nigeria and their growth, productivity and nutrient were monitored. Tadpoles were harvested, processed and used in compounding five experimental diets of 42.5% crude protein to replace fish meal at different inclusion levels of 0% (control), 25, 50, 75 and 100% and fed to *Clarias gariepinus* fingerlings. The mean weight gain for cultured tadpoles was 3.16 g/tadpole; specific growth rate 2.21% and feed conversion ratio, 1.04. The result of the feeding experiment showed that WG, RGW, SGR, FCR and feed intake favored fingerlings fed 50 and 25% whole tadpole meal including diets with insignificant difference p< 0.05 compared to other treatments. Highest significant difference p< 0.05 for crude protein digestibility of 90.88% was for 25% whole tadpole meal. Better cost benefit ratio was reported in 25 and 50% whole tadpole meal diets. Based on this result, the replacement of tadpole meal is recommended for 25 and 50% inclusion levels in the diet of *Clarias gariepinus* for better growth performance, feed utilization, health status and cost benefits.

Key words: *Bufo maculate, Clarias gariepinus* and crude protein.

INTRODUCTION

The cost of feeding is a major factor affecting the development of aquaculture enterprise in Nigeria (Ayinla *et al*., 2006). On this account, Falaye, (2006) and Fagbenro *et al*. (2008) reported that the use of commercial pellets and supplementary fish feed accounts for about 60 and 40%, respectively of the recurrent cost of fish farming venture in Nigeria. In 1989, the presidential task force on alternative formulation of feed identified feeding of fish as one of the major problems facing aquaculture industry development in Nigeria and proffered possible solution geared towards increasing fish production from this sector. One of the suggestions made by this task force was on the utilization of non-conventional proteins supplements of both animals and plants origin in practical fish diet and the following alternative feed ingredients: grasshopper, garden snail, shrimps head, feather, termite, maggot, crab meal, defatted cocoa cake, yam peel, mussels, periwinkle, lizard, palm grub, earthworm and frog amongst others (Fagbenro, 2005; Idonibuoye-obu *et al*., 2003; Ajah, 2005; Alegbeleye and Oresegun,2009) had been studied as both protein and energy sources in fish feed.

Most fish farmers in Nigeria lack information on how to assess and determine the profitability of their farms. The consequences are that many fish farmers do not achieve high profit from their ponds. Other 'potential' farmers avoid going into fish farming and other farmers become 'inactive' because the profitability of aquaculture has not been demonstrated to them. It has also been observed that most fish farmers do not keep records on their fish farming activities and if they do, it is done in a haphazard manner. Yet, record keeping is one of the most fundamental and critical aspects of aquaculture, in evaluating the viability and profitability of any aquaculture enterprise. There is therefore the need to formulate a basic tool that farmers can use as a guide in keeping farm records and evaluating the profitability of their fish farms. Fisheries occupy a unique position in the agricultural sector of the Nigerian economy. In terms of gross domestic product (GDP), the fisheries sub-sector has recorded the fastest growth rate in agriculture to the GDP. The contribution of the fisheries sub-sector to agriculture GDP was estimated as 4.0% in the year 2007, out of the total estimate of 40% being contributed by agriculture to GDP (CAN, 2010).The Food and Agricultural Organization of the United Nations (FAO, 2006) stated that Nigeria is a protein-deficient country. The protein deficiency in the diet can be primarily remedied through the consumption of either protein-rich plant or animal foodstuffs. Protein from animal sources is in short supply in Nigeria due to the rapid increase in human population annually as well as the decrease in livestock population due to several factors including diseases, desertification, drought, climate change, global warming, scarcity and high cost of quality feeds, poor genetic qualities, limited supply of indigenous breeds and avian flu disease (H_5N-1) which brought about mass mortality of poultry. These factors have raised the cost of animal protein to a level that is almost beyond the reach of the ordinary citizen. This situation therefore has given rise to a considerable increase in the demand for fish to supplement the needed animal protein intake. Nigerians are large consumers of fish with demand estimate at 1.4 million metric tons. However, a demand –supply gap of at least 0.7 million metric tons exists nationally with import making up the short fall at a cost of almost 0.5 billion US dollars per year. Domestic fish production of about 500,000 metric tons is supplied by artisan fisher-folk (85%), despite overfishing in many water bodies across the country (Adeyemo *et al*., 2010). Apart from high availability and relatively cheap cost, there is hardly any religious taboo and any known cultural limitations affecting the consumption of fish unlike pork and beef meat. Apart from its nutritional importance, fish also add varieties and taste to diets as well as improved palatability of food. Nigeria has a land area of 923,768km^2 with a continental shelf area of 47,934km^2 and the length of coast line of 853km. it also has a vast network of inland waters like rivers, flood plains, natural and man-made lakes and reservoirs (Lovell, 2008). The inland waters mass was estimated to be about 12.5 million hectares of inland waters capable of producing 512,000 metric tons of fish annually (Adikwu, 2003; Ajah, 2005).

Study significance

Bufo maculata is the commonest species from the family Bufonidae found in the semi arid zone of Nigeria (Akegbejo, 2007). This animal with very high reproductive rate does breed during the rainy season alone in the wild and hardly is there any competition for this resource by man in this part of the world. For proper utilization of this natural resource and limitation of its annual wastage, it could be diverted as fishmeal supplement in fish feed. The possibility of replacing fish meal with toad meal has been reported by Annune, (2008). There is dearth of information on the culture, nutritive value and utilization of *B. maculata* as fish feed ingredients in literature hence there is a need for this study. The utilization of the tadpole stage will also go a long way to control the population of this animal.

FISH FOOD AND LIVE FEED FOR FISH LARVAE

The transition from endogenous to exogenous feeding is a critical event in the life of a fish. It is generally acknowledged that live feed during the first few days of hatching is necessary to ensure adequate lava survival Dry artificial feeds are inadequate to nourish small larvae during the first stages of feeding. Such feeds can be used successfully after feeding the larva with live feed for some time. Fish larvae fed with live feed in the wild or cultured have higher survival rate than those fed with artificial feeds (Guerrero, 2010). Live feed organisms include zooplanktons. These are the rotifers, copepods, cladoceras and other larval and adult forms of some invertebrates. The type of fish feed determines fish production. The use of live feed in fish culture, have received tremendous attention in countries where fish culture is well developed. For example, in Malaysia, feeding with fusoria and rotifer, start from the second day of post hatch and lasts for one week (Houlihan *et al*., 2001). Feeding of larvae with Daphnia, a cladocera was also successful in Hong Kong. *Cultured moina* (cladocera) in combination with rotifer are used effectively in Singapore. The Nagasaki prefectoral institute of fisheries in Japan designed a production technique for the culture of rotifers on suitable phytoplankton substrate (*Chlorella* sp.) and baker's yeast for larvaerearing. The mass production of marine fish fry in Japan depended on the continuous supply of rotifer (*Branchionus plicatilis*)

(Lovell, 2008). These Japanese required 30 billion rotifers to raise one million pagrus major to 10 mm long. Successful rearing of milkfish (*Chanos chanos*) larvae also depends almost entirely on the use of the live feed, rotifer (*Branchinous plicatilus*).The African Regional Aquaculture center, Port Harcourt, reared the larvae of *Clarias gariepinus*, *Heterobranchus bidorsalis* and Heteroclarias with live feed, Moina spp cultured in earthen ponds. The most widely cultured feed are rotifers because of their abundance in any water body. Monia and Daphnia species are also widely cultured (Robert, 2009). Live feeds can be cultured using both organic and inorganic fertilizers. These fertilize the medium to produce a phytoplankton bloom. The desired species of zooplankton for the culture are later introduced into the medium. The use of inorganic fertilizer (NPK and urea mixture) can be effective for the culture of *Moina* sp. A shed of 3 m × 4 m constructed and plastic bowels of 40 L capacity is preferable for the culture. Different mixtures can be used. Moina species seem to grow best in 0.5 g urea and 0.5 g NPK and increases in growth rate within few days of inoculation (Roberts, 2003).Live feeds can also be cultured using concrete tanks covered with transparent nylon sheets. The nylon sheet allows light to pass through the concrete tank. The water can be fertilized with chicken manure or cow dung mixture. The ratio of 31:7 of chicken manure and cow dung is recommended. Algal bloom appears after fertilization, followed by zooplankton, in most cases, the rotifer; *Asplanchna priodonta* appears after the inoculation. An abundance of 100,000 organisms per liter can be observed at the peak of the bloom (Devendra, 2009).

FISH FEED INGREDIENTS

Most fish farmers and ornamental fish hobbyists buy the bulk of their feed from commercial manufacturers. However, small quantities of specialized feeds are often needed for experimental purposes, feeding difficult to maintain aquarium fishes, larval or small juvenile fishes, brood fish conditioning, or administering medication to sick fish. In particular, small ornamental fish farms with an assortment of fish require small amounts of various diets with particular ingredients. It is not cost effective for commercial manufacturers to produce very small quantities of specialized feeds. Most feed mills will only produce custom formulations in quantities of more than one ton and medicated feeds are usually sold in 50 pound bags. Small fish farmers, hobbyists, and laboratory technicians are, therefore, left with the option of buying large quantities of expensive feed, which often goes to waste. Small quantities of fish feed can be made quite easily in the laboratory, classroom or at home, with common ingredients and simple kitchen or laboratory equipment.

Nutrients essential to fish are the same as those required by most other animals. These include water, proteins (amino acids), lipids (fats, oils, fatty acids), carbohydrates (sugars, starch), vitamins and minerals. In addition, pigments (carotenoids) are commonly added to the diet of salmonid and ornamental “aquarium” fishes to enhance their flesh and skin coloration, respectively.

In their natural environment fish have developed a wide variety of feeding specializations (behavioral, morphological, and physiological) to acquire essential nutrients and utilize varied food sources. Based on their primary diet fish are classified as carnivorous (consuming largely animal material), herbivorous (consuming primarily plant and algae), or omnivorous (having a diet based on both plant and animal materials). However, regardless of their feeding classification, in captivity fish can be taught to readily accept various prepared foods which contain the necessary nutrients. Increased understanding of the nutritional requirements for various fish species and technological advances in feed manufacturing, have allowed the development and use of manufactured or artificialdiets (formulated feeds) to supplement or to replace natural feeds in the aquaculture industry. Abundant supplies of feedstuffs are available, and farmers and hobbyists are now able to prepare their own fish feeds from locally available ingredients (Annune, 2008).

Some common conventional feed stuff

The feed industry is, currently maintained by conventional ingredients earlier mentioned. This is in spite of the attendant vagaries in high cost and scarcity, creating the existing problems that are apparent in high cost of feed (Robinson and Brunson, 2008). Examples of such feedstuff include:

Groundnut cake

This contains about 45% crude protein but lacks the essential amino acid, lysine. When moldy, it becomes poisonous due to the presence of the mycotoxin called aflatoxin.

Soybean meal

This feedstuff is fast gaining increasing acceptability and use in the feed industry. It has a balanced amino acid profile and can replace a substantial part of fishmeal. The use of this feed stuff is however limited due to its high fat content and presence of trypsin inhibitor.

Palm kernel meal

This contains a fairly high quantity of are only useful when its crude fiber content is high.

Brewers dried yeast

This is a byproduct of the brewery industry. It contains sufficient quantity of crude protein but limited in amino acids, methionine and cystine.

Brewers dried grain

This is readily available and contains similar protein levels as palm kernel cake. The crude fiber content is high and therefore in limited use.

Maize

Maize is palatable and free from antinutritional factors. The energy content is high. This limits use in fish feed.

Wheat offal

The nutritional property of wheat Offal is similar to palm kernel meals. The two can therefore be used interchangeably but scarcely together. Wheat Offal is very scarce due to adverse government policy.

Fish meal

Fish feed is hardly formulated without fish meal. Apart from its high protein content, fishmeal also acts as an attractant. Fishmeal is produced either from the trash obtained from trawling or fish waste from the canning industry. The percentage protein depends on the source of fish product and method used in producing the fishmeal.

Poultry byproduct meal

Poultry processing generates a lot of wastes such as offal, blood and heads of birds. These wastes can be processed to form poultry byproduct meal. The protein content is high with a balanced amino acid profile. It can replace fishmeal without any adverse effect on the fish. The quantity and proportion in which these conventional feedstuffs are used depend on its nutrient composition, presence of ant nutritional substances, palatability and cost (Robert, 2003). The maximum and minimum levels are also recommended as these levels are adjustable upwards or downwards. The inclusion of mineral vitamin mixture is important due to its nutritional implication. Mineral vitamin mixture is added to the final feed, because fish oil and other unsaturated oils used in feed formulatio n soon becomes unpalatable. When eaten, this can result in pathological symptoms in the fish (Robert, 2003).

Unconventional feeds for culture fish

Unconventional fish feeds are potential feed ingredients, which have hitherto not been used in fish feed production for the reasons that:
(i) they are not well known or understood.
(ii) no effective study of the method of production with a view to commercializing them.
(iii) they are not readily available.
(iv) they can be toxic or poisonous.

These feeds are generally referred to as unconventional feed ingredients. They contain high quality feed ingredients that can compare favorably with conventional feed types. They are expected to be cheaper by virtue of the fact that there is no competition for human consumption. Unconventional fish feed can be of animal or plant source (Roberts, 2003).

Animal source

These are feed from any living thing, other than human being, that can feel and move. Examples include tadpole meal; fly larvae, earthworm meal, toad meal, shrimp waste, crab meal and animal wastes such as pig and poultry droppings and blood meal (Cochran, 2009).

Tadpole meal

Frogs and toads breed at the onset of the rainy season with the first rains acting as stimulus for reproduction. Eggs are laid in stagnant pools or any body of water and later hatch into tadpoles. Tadpoles are seen swimming and feeding from food obtained in the pool of water. There is no parental care for these tadpoles. They however survive until the pool either dries up or they metamorphose into adult frogs or toads in 23 months. Because of the period of life cycle spent in water, tadpoles can be cultured like fish and harvested before they can metamorphose. The harvested tadpoles can be processed by oven drying or smoking over a kiln. For immediate use, they can be feed whole to adult fish or pulverized and added to other feed ingredients at 4050% depending on availability. Proximate analysis showed that the meal contained 50% crude protein. A direct comparison with fishmeal in a replacement trial on the catfish *Heterobranchus bidorsalis* showed that it was more cost effective, though fishmeal was nutritionally superior. It can however be used in place of fishmeal (Daly, 2005).

House fly larvae (Musca domestica)

When there is a supply of damp decaying organic matter, houseflies thrive. It serves both as food and breeding ground for the adults and sustenance for the resultant larvae. In fact, within a short period of the existence of such matter, large number of fly eggs became apparent. A simulation of such condition is achieved by creating a decaying organic matter with an attractant such as a combination of fish on trays or shrimp waste with finely ground maize or soybeans, groundnut cake or palm kernel cake. The mixture is turned into a slummy watery waste with water in a drum one quarter or half filled and left open in the field. Harvesting of maggots can commence from the third day. A continuous check can be made to see the fly larvae, which metamorphose from the laid eggs. When the quantity of wriggling larvae (maggots) is satisfactorily high, the paste is diluted and larvae harvested with a fine mesh sieve, washed thoroughly to remove the substrate and allowed to drip dry (De Silva and Anderson, 2005).
Processing follows by:

(i) oven drying
(ii) smoking over a kiln
(iii) pulverising with a pepper grinder and mixing with other fresh ingredients
(iv) they can be fed whole to fish
(v) they can be processed with the substrate in the mixed form instead of separate harvesting.

The nutrient quality analysis of processed larvae is Moisture 8%, protein 45%, fat 15%, Ash 8% and chitin 25% (Houlihan *et al*., 2001).

Animal wastes

Faeces from animals, particularly piggery and poultry droppings can be used as pond organic fertilizers for the stimulation of plankton growth. However, both animal droppings are used as a direct source of food to fish. As direct food for fish; pig wastes collected early in the morning without contamination by pig urine, are dropped into a marked area of the pond as food for the fish. The same thing goes for poultry droppings. In fact some fish culturist use poultry droppings without further use of any artificial feed with good results. However, care must be taken to avoid pond contamination. As after fermentation and build up of fly larvae; the droppings can be left in open containers for a few days to allow fermentation and build up of larvae. The larvae and droppings are let into the ponds. As oven drying and incorporation into feeds; the dropping can be oven dried or sun dried during the harmattan period and added to other ingredients in fish feed. Such droppings are known to contain nearly 30% crude protein content. Animal wastes are particularly useful in the poly culture of the local catfishes and tilapias (Daly, 2005).

Earthworm meal (Lumbricus terrestris and Allobophora long)

These are detritivorous terrestrial oligocheate worms. They live in the soil and feed on decaying leaves and other organic matter, which they later pass out as worm caste. In other words, they convert organic matter to soil. In this regard they are used in the breakdown and utilization of human and animal waste. These worms are hermaphrodites and reproduce while being used to break down detritus increasing their number at the same time. They are commercially produced by, heaping animal, human wastes or refuge in a land with enough moisture in the soil or swamps. Suitable pairs of earthworms are introduced. These would breed with the detritus serving as source of nutrients to them. Harvesting can be done after six months. This is by digging up lumps of earth and gently breaking them up to release the worms. Processing is either by oven drying, smoking over a kiln or pulverising with a pepper grinder. The best known use of earthworm today, is as fish bait in artisan fisheries. Proteins and essential amino acid content of earthworm is shown as reported by Henken *et al*., (2007).

Toad meal *(Bufo regularis)*

Toads in the tropics are seen in moist or damp areas in the forests, house surroundings etc. In the dry season, they move under stones. They breed by laying large number of eggs in pools of water during the rains, which hatch into tadpoles. The tadpoles metamorphose into toads. Therefore, it is possible to breed toads on a commercial scale and processed by oven drying. Toads are however, viewed with revulsion and people seldom touch it because of its moist rough skin. Some fish culturist, kill toads and put into the fishpond. When the toads go putrid, the catfishes feed on it. Putrefaction or fermentation removes the poison in the toad skin. Nutritionally, toads contain 99% protein and compares favorably with fish meal Winfree, (2002).

Blood meal

Cow blood is available in slaughter houses on daily basis. This can be obtained freely. Processing is, by boiling the blood followed by:
(i) dry in an oven or smoke over a kiln
(ii) sundry, particularly, during the harmattan. The bulk of commercial blood meal is processed during the dry season.

Add straight into the mixture of well ground ingredients. Dry cassava powder can be used as a binder and boiling water to gelatinize it. Mix thoroughly and spread out to dry. The result is a feed with crumble consistency ideal for adult fish and fingerlings. Blood meal is high in protein content (85%). It is a supplemental source of lysine but low in methione. Antagonism exists between leucine and isoleucine which renders the latter unavailable if combined with soybean meal, the result is ideal for fish growth.

Plant sources

Plants are nonmotile multicellular organisms whose cells have cellulose cell walls and chloroplasts. The chlorophyll in the chloroplasts enables plants to make their own food by photosynthesis. Plants include *Thallophyta*, *Bryophyta* and Tracheophyta. Thallophytes and bryophytes do not have any stem for conducting food and water from one part of the plant to another. This restricts their size. Tracheophytes have conducting systems, so they are much larger. The plant sources of fish diets however include, leaf protein, leaf meal, aquatic macrophytes, cultivable pulses such as mucuna bean, yam beans, bread beans, winged beans or any legume ornamental that can yield pods with seeds. The mixture is allowed to settle in separating flax for a period of 12 h. The bottom slurry is protein obtained by decanting and drying. Any leave is suitable for this method. The resulting powder is leaf protein concentrate containing up to 1550% crude protein (AOAC, 2000).

Fish Feed Formulation

Feeds are formulated to be dry, with a final moisture content of 610%, semi moist with 3540% water or wet with 5070% water content. Most feeds used in intensive production systems or in home aquaria are commercially produced as dry feeds. Dry feeds may consist of simple loose mixtures of dry ingredients, such as “mash or meals”, to more complex compressed pellets or granules. Pellets are often broken into smaller sizes known as crumbles. The pellets or granules can be made by cooking with steam or by extrusion. Depending on the feeding requirements of the fish, pellets can be made to sink or float (Alegbeleye and Oresegun, 2009).

Flakes are another form of dry food and a popular diet for aquarium fishes. Flakes consist of a complex mixture of ingredients, including pigments. These are made into slurry which is cooked and rolled over drums heated by steam. Semi moist and wet feeds are made from single or mixed ingredients, such as trash fish or cooked legumes, and can be shaped into cakes or balls. Feed formulation is an act and its knowledge allows the formulator to take advantage of substituting one feedstuff for another and compound feed at the lowest cost (Falaye, 2006).

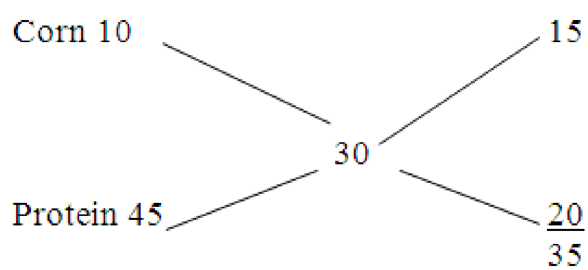
The fish however, is the subject to evaluate the nutritional adequacy of the feed in terms of increase in weight gain, higher reproductive capacity or reduced mortality and morbidity (CAN, 2010). There is no single way for the preparation of formulated fish feeds, however, most methods begin with the formation of a dough like mixture of ingredients. Ingredients can be obtained from feed stores, grocery stores, pharmacies, and specialty stores such as natural food stores, as well as from various companies that may be found through the internet. The dough is started with blends of dry ingredients which are finely ground and mixed. The dough is then kneaded and water is added to produce the desired consistency for whatever fish is going to be fed. The same dough may be used to feed several types of fish, such as eels and small aquarium fish. Pelleting or rolling converts the dough into pellets or flakes, respectively. The amount of water, pressure, friction, and heat greatly affects pellet and flake quality. For example, excess water in the mixture results in a soft pellet. Too little moisture and the pellet will crumble. Proteins and especially vitamins are seriously affected by high temperatures. Therefore, avoid storing diet ingredients at temperatures at or above 70ºC (158ºF) and do not prepare dry feeds with water at temperature higher than 92ºC (198ºF). Making your own fish feed requires few specialized tools. The tools are used primarily for chopping, weighing, measuring ingredients, and for blending, forming and drying the feed. Most of the utensils needed will already be in the laboratory or kitchen. Multipurpose kitchen shears, hand graters, a paring knife, a 5 inch serrated knife, a 6 to 8 inch narrow blade utility knife, and a 10 inch chef knife for cutting, slicing, and peeling can be used. A couple of plastic cutting boards protect the counter and facilitate handling the raw ingredients. Heat resistant rubber spatulas, wooden and slotted spoons, long handled forks, and tongs are very good for handling and mixing ingredients. A basic mortar and pestle, electric blender, food processor or coffee grinder are very useful to chop or puree ingredients; use grinder sieves and mince die plates to produce the smallest particle size possible. A food mill and strainer such as a colander or flour sifter help discard coarse material and obtain fine food particles. For weighing and measuring ingredients, dry and liquid measuring cups and spoons, and a food or laboratory bench scale are required. Other utensils include plastic bowls (1½, 3, 5, and 8 quarts) for weighing and mixing ingredients, a thermometer, and a timer. A 3-quart saucepan and 10inch stockpot are good for heating gelatins and cooking raw foods such as vegetables and starches. The ingredients and blends may be cooked in a small electric or gas burner. A few trivets to put under hot pans will protect counters and table tops (Lovell, 2008).Ingredients may be mixed by hand using a rotary beater or wire whisk, however, an electric mixer or food processor is more efficient. After mixing, a dough is formed which can be fashioned into different shapes. A pasta maker, food or meat grinder will extrude the dough into noodles or “spaghetti” of different diameters. As the noodles emerge from the outside surface of the die, they can be cut off with a knife to the desired length or crumbled by hand, thus making pellets. A potato ricer also serves to extrude the dough into noodles of the same size. For making flakes, a traditional hand cranked or electric pasta maker will press out the dough into thin sheets. The pellets or thin sheets can be placed on a cookie sheet and dried in a household oven on low heat or in a forced air oven. A small food dehydrator also performs the task quite well. To add extra oil and/or pigments to pellets, a handheld oil atomizer or sprayer can is useful. To separate pellets into different sizes, a set of sieves (e.g., 0.5, 0.8, 1.0, 2.0 and 3.0 mm) is required. Freezer bags serve to store the prepared feeds, and using a bag vacuum sealer will greatly extend the shelf life of both ingredients and the feed. The feed can be stored double bagged in the freezer but should be discarded after 6 months. Ideally, dried larval feeds are not frozen but stored in the refrigerator for no longer than 3 months (Adikwu, 2003). A finished diet, especially used for experimental purposes, should be analyzed for nutrient content (proximate analysis: crude protein, energy, moisture, etc.). In addition, anyone intending to make his/her own fish feeds with unfamiliar ingredients should have them analyzed prior to their use. Purified and semi purified diets are used primarily in experimental formulations to study the effects a nutrient, such as the amount or type of protein, may have on the health and growth of fish. One simple formulation, which is used traditionally to feed ornamental fish in ponds, consists of a mixture of 30% ground and processed oats or wheat and 50% of fish meal or pellets from a commercial manufacturer. By weight, approximately 23% of fish oil, and a 0.3% vitamin and a 1% mineral premix are added to the mixture. This mixture is blended with water and can be formed into dough balls of different sizes. A semi purified diet, determines the optimum protein level required (Harborne, 2000). This diet also can be used as a basis for feeding other types of ornamental fish in the laboratory. The cichlid feed recipe was derived principally from salmonid formulations and uses casein as the purified protein source. The ingredients in the recipe are listed under major nutrient categories such as proteins, carbohydrates, lipids, vitamins, and minerals. Pigments are added to enhance the coloration of ornamental fish gelatin-based diet developed for difficult to feed fishes by the National Aquarium in Baltimore. In this diet, gelatin is the primary binder. This recipe can be modified and supplemented with a variety of ingredients. Supplemental or replacement ingredients are presented. Gelatin based diets are popular in the aquarium fish industry and useful for preparing medicated feeds at home. Feeds are compounded by the use of the popular “Pearson square method”. In this method:

(i) put the ingredients on the left side.
(ii) put the required protein level at the center.
(iii) subtract each quantity from the required feed weight that would give protein level.
(iv) substitute to get the answer.

For example,using two ingredients to achieve the required 30% protein level.

Method

Corn = 10% protein
Shrimp = 45% protein
Required protein level = 30%
Therefore, 15/35×100 = 42-85 kg, 20/35×100 = 57.14 kg



Cross checking

10/100×42.85 = 4.285
45/100×57.14 = 25.713
By adding, 4.285+25.713 = 29.998% = 30%.

Feeding methods

The commonest method of providing food for the culture fish is to fertilize the culture media with organic and inorganic fertilizers to stimulate the growth of phytoplankton and zooplanktons. Other invertebrates such as water insects and their larva; benthic invertebrates and detritus materials are also utilized as food for fish (Winfree, 2002).In a poly culture system where different species of fish are grown together, the fry and fingerlings of herbivorous fish are, preyed upon by carnivores. With increased growth rate of the stocked fish, the available natural food in the pond become inadequate to support the fish population, hence, the need to supplement the natural food with artificial feeds for enhanced fish growth (Winfree, 2002).

The selection of fish for culture with supplementary diet depends on the crude protein requirement of the fish and the unit cost of the feed ingredient available in a given locality.

This consideration is important in view of the fact that fish feed account for between 4070% of the operating cost of a fish farm with an intensive management system (Winfree, 2002).

Feeding is one of the most important aspects of the fish culture. The basic activities of the fish include: Its growth, development and reproduction. All these take place at the expense of the energy, which enters the fish in the form of its food (Madu *et al.,* 2003).

MATERIALS AND METHODS

This experiment would be conducted within Faculty of Science complex, University of Abuja, Nigeria (Lat 9° 10' 32"N: Lon 7° 10' 5°"E). Annual rainfall ranges from 1800-3000mm while the maximum day temperatures range from 27.5°C to 30.9°C.

Procurement and analysis of feed ingredients

All feed ingredients to be used in the preparation of the fe ed formulae would be purchased from local markets within and around Gwagwalada, F.C.T, Abuja, Nigeria. Tadpole meal was collected in raw form, washed with tap water and converted into meal after sun drying and grinding. All formulated feeds were analyzed for proximate composition i.e., percentage of protein, fat, moisture, ash, fiber and energy (Kcal/100g) following the methods of AOAC, (2000) and Akegbejo-Samson, (2007). Each determination was done in triplicate and averaged was taken.

Preparation of experimental diets

Harvested tadpole was processed into dry meal and grounded to powered form, analyzed for proximate composition and used as substitute for fish meal at inclusion levels of 0, 25, 50, and 75% to prepare four isonitrogenous **(**crude protein 42.5% and Isocaloric (gross energy-1900 kcal kg^{-1}) diets (TP_1 (control) TP_4). These were compounded and fed 5% body weight to fingerlings of *Claris gariepinus* at a stocking density of 20 fingerlings per 50 L plastic tanks in indoor mini-flow though system of the hatchery unit, for 6 weeks. Each experimental treatment was in triplicate. Each treatment would also be monitored for mortality, dead fish would be removed, counted, recorded and use in determining the survival rate (Adikwu, 2003).

Procurement of fish

C.gariepinus fingerlings were procured from Agricultural Development Project, (ADP) Gwagwalada, Abuja, Nigeria. *C. gariepinus* fingerlings was stocked in 50L plastic tanks, and acclimatized for a period of 15 d ays. During acclimatization fingerlings was fed with control diet at 3% body weight twice a day.

Experimental design

This experiment was laid out in complete randomized design with 4 treatment and 3 replicates.

Physicochemical parameters

On daily basis water temperature, dissolved oxygen and pH would be monitored. Temperature would be recorded using digital thermometer (IR Thermometer Smart Sensor), DO (dissolved oxygen) would also be recorded using DO meter (HANNA-HI 9145) and pH would be determined using pH meter (WTW D82362 Wellheim, Germany). Phosphate, nitrate, nitrite, total ammonia, chloride, total alkalinity and total hardness would be measured following standard methods (AOAC, 2000).

Growth indices

Total WG, percent WG, SGR, FCR and survival rate were calculated according to Idonibuoye-obu *et al*. 2003, SGR according to Houlihan *et al*. 2001 and FCR according to Henken *et al*., 2007.

Statistical analysis

All the data would be analyzed using analysis of variance (ANOVA) and mean will be compared using Duncan multiple range test at 5% level of significant.

RESULTS AND DISCUSSION

The three-inclusion level of tadpole meal in the experimental feed supported the growth for *Clarias gariepinus.* However, growth performance and feed utilization was favored by low inclusion level of tadpole meal in the experimental feed. This result is similar to the report of several authors who have demonstrated the use of several species of tadpole as a partial replacement for fishmeal in the diet of fish and other animals. Fagbenro *et al*. (2008) reported the use of tadpole meal *Buffalo maculeta* in the diet of the Nile Tilapia *(Oreochromis*

Table 1: Percentage composition of ingredients and production cost of whole tadpole meal-based diets for the feeding trial.

Ingredient	TP0 (control)	TP1 (25%)	TP2 (50%)	TP3 (75%)
Fish meal	0.00	22	15	7.5
Tadpole meal	0.00	25	50	75
Yellow maize	00.0	21.10	15.20	8.9
Groundnut cake	00.00	17.30	20.90	24.60
Soybean meal	00.00	14.60	16.90	19.50
Blood meal	00.00	10.00	10.00	10.00
Chromic oxide	0.00	0.50	0.50	0.50
Vitamins/minerals	0.00	2.00	2.00	2.00
Palm oil	0.00	2.00	2.00	2.00
Bone	0.00	1.00	1.00	1.00
Common salt	0.00	0.50	0.50	0.50
Cassava starch binder	0.00	1.00	1.00	1.00
Total	00.00	100.00	100.00	100.0
Calculated crude protein (%)	00.00	42.50	42.50	42.50
Calculated gross energy (Kj/100g)	1900.00	1900.00	1900.00	1900.00

niloticus). They stated that fish fed tadpole based diet had higher growth rates than fish fed diet containing water fern meals. The authors indicated the possibility of partial replacement of fishmeal with tadpole in the diet of Nile Tilapia. Houlihan *et al*. (2001) reported that grass carp performance on a tadpole diet was superior to fish maintained on catfish chow. Adeyemo *et al.* (2010) fed channel catfish on prepared diet consisting of 20% dry tadpole; the weight gain, food conversion and energy use were equal to central diets (a standard catfish feed). Zeitler *et al*. (2008) reported also the replacement of sesame oil cake by tadpole in broiler diet. They stated that partial replacement of the costly oil seed by cheaper unconventional tadpole in broiler diet resulted in increase profitability.

The protein efficiency ratio in the diets at 25% to 50% inclusive levels of duckweed meal in the experimental diet showed no significant difference compared with the control diet. Tom and Vannostrand, (2009) reported that 30% fermented lemna leaf meal incorporated in the diet of *Labeo rohita* gave the best performance in terms of growth response, food conversion ratio and protein efficiency. From this result, 25% duckweed meal diet had the best specific growth rate and food conversation ratio.

Fagbenro *et al.* (2008) reported that there was no significant difference in growth performance and nutrient utilization of fish fed on diets containing up to 20% tadpole inclusion and the control. They however, stated that increase in dietary tadpole inclusion resulted in progressively reduced growth performance and nutrients utilization of fish. This report is similar to the findings of this study. Inclusion of tadpole meal in the diet of other animals to replace fishmeal or soybean has also been reported by Devendra (2009), Guerrero, (2010) and Oresegun and Alegbeleye, (2011). Ayinla *et al.* (2006) reported no significant difference between the growth performance of fish that were fed diets containing up to 50% tadpole and fish that were fed the control diet, while carcass lipid and carcass protein also increased except for the diet with 75% tadpole meal in the common carp, *Cyprinus carpio*, fry. These authors concluded that a diet containing up to 25% tadpole means could be used as a complete replacement of fishmeal for commercial feed in the diet formulation for common carp fry.

The basic nutrient that cannot be compromised in the choice of ingredients for feed formulation and preparation is protein (Zeitler *et al*., 2008) and since each of the experimental diet supplied the optimum required amount they were adequately utilization by the fish. The lipid content of each experimental diet increased with increase in the proportion of the tested animal protein supplements (Table 2). This lipid increase may have had a sparing effect on the dietary protein and complement its utilization (Adeyemo *et al*., 2010). This observation corroborate with that of NRC, (2003), that practical diets should be formulated not only to meet the optimum ratio of protein

Table 2. Proximate and energy composition of whole tadpole meal.

	Tadpole meals			
Composition	0%	25%	50%	75%
Crude protein	47.60^{a}	47.01^{a}	46.31^{b}	43.16^{b}
Ether extract	11.50^{a}	10.91^{ab}	13.10^{c}	14.66^{cd}
Crude fibre	33.15^{c}	3.17^{a}	3.74^{a}	3.92^{ab}
Ash	4.11^{a}	9.47^{b}	11.10^{c}	12.92^{d}
Nitrogen free extract	3.16^{a}	19.47^{cd}	13.25^{c}	10.51^{b}
Dry matters	98.2^{d}	82.28^{a}	84.36^{b}	85.16^{c}
Gross energy Kcal/100g	1504.30^{a}	1732.04^{b}	1732.90^{b}	1743.17^{c}

All value on the same row with different superscripts are significantly difference $p<0.05$.

Table 3. Growth indices of *Clarias gariepinus* fingerlings for whole tadpole meal diets for 70days.

Indices	TP_0	TP_1	TP_2	TP_3
Inclusion level (%)	0	25	50	75
Mean initial weight (g fish^{-1})	1.82±0.02	1.81±0.06	1.84±0.04	1.88±0.07
Initial total length (cm fish^{-1})	6.4^{b}	6.2^{a}	6.6b^{c}	6.4^{b}
Mean final weight (g fish^{-1})	9.01^{c}	9.50^{cd}	8.75^{b}	8.21^{a}
Relative growth rate (%)	323.6^{c}	406.7^{d}	223.1^{b}	112.1^{a}
Specific growth rate (%)	0.92^{c}	1.80^{cd}	0.89^{b}	0.67^{a}
Condition factor	1.30^{c}	1.24^{b}	1.38^{c}	1.12^{a}
Survival (%)	88.1^{b}	92.2^{c}	86.2^{a}	71.7^{b}
Feed conversion rate (%)	2.33b^{c}	3.42^{c}	2.12^{b}	1.08^{a}
Protein retention (%)	51.30^{d}	45.12^{c}	41.04^{b}	25.43^{a}
Protein efficiency rate	0.97^{c}	0.99^{cd}	0.87^{b}	0.76^{a}
Incidence of cost	1.94^{c}	1.82^{b}	1.96^{c}	1.77^{a}

All value on the same row with different superscripts are significantly difference $p<0.05$.

to energy but also the adequate amount of lipid needed by the fish (Tables 1, 2, 3 and 4). Dietary lipids also provide essential polyunsaturated fatty acid for normal growth and development of cells and tissues (Henken *et al*., 2001). Among the experimental diets studied, tadpole meal diets were rancid ealier and more microbial growths at both 8°C and room temperature (28-30°C) storage temperatures than the other diets. The reason for this would have been their high lipid concentration compared to the other diets. This agrees with the report of Lovell (2008) that the major problem faced by animal feed compounders is the susceptibility of individual ingredients and formulated diets having high lipid and moisture to oxidative damage or oxidative rancidity and microbial attack.

Despite the fact that the studied diets were dried which eliminate high moisture; deterioration were recorded from physical, chemical and microbial studies which showed that moisture was not a major factor in feed spoilage (De Silva and Anderson, 2005 and Sogbesan, 2006). The reduction in lipid and protein composition from all the experimental diets at the end of the experimental period

Table 4. Physicochemical parameters (Mean ± S.E) of water during experimental Trial in *Clarias gariepinus.*

Treatments	Temp (C)	DO (mgl^{-1})	pH	Phosphate (mg^{-l})	Nitrate (mg^{-l})	Ammonia (mg^{-l})
TP0 Control	21.90±1.1	7.02±0.3	7.63±0.6	2.11±0.0	8.90±0.2	0.04±0.0
TP1 (25%)	22.21±1.6	6.92±0.0	7.69±0.1	2.00±0.0	8.94±0.3	0.03±0.0
TP2 (50%)	22.56±2.4	6.86±0.9	7.76±0.9	2.18±0.0	9.84±0.3	0.05±0.0
TP3 (75%)	22.27±1.5	6.87±0.3	7.74±0.5	2.18±0.0	7.52±0.6	0.03±0.0

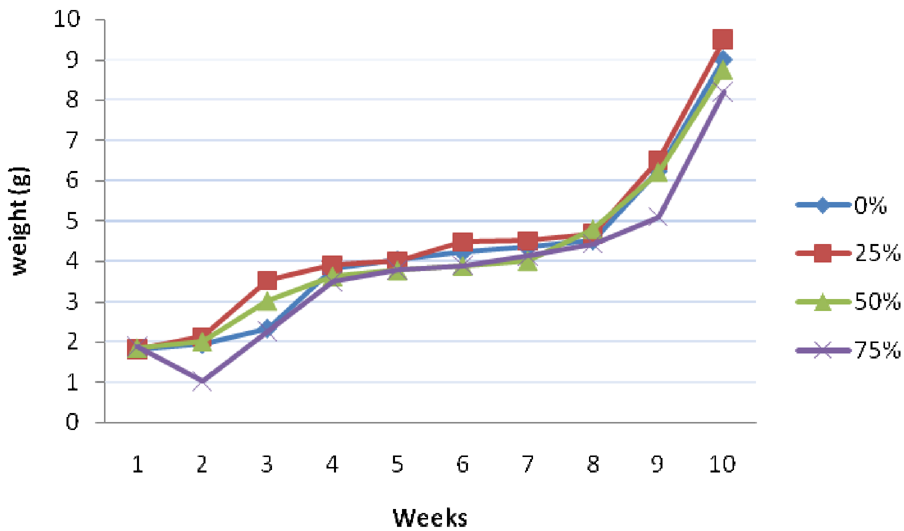


Figure 1. Growth pattern of *Clarias gariepinus* fed whole meal tadpole meal based diet for 70days.

corroborate the reports of De Silva and Anderson (2005) and Henken *et al.* (2007) that within a period of 2-4months feeds are prone to reduction in their major nutrient composition. Reduction of nutrient content from each diet at the end of the experiment could have been as a result of increase in activity and microbial population, solubilization of minerals into weak acids, other oxides, temperature and humidity fluctuation as also reported by (Benitez-Mandryano, 2012). The result of the microbiological analysis showed that the method of storage had effect on microbial growth though lower microbial load were recorded from 8°C compared to those preserved at room temperature. Wilson *et al.* (2009) and Cochran (2009) documented that cold storage is the most effective way of preserving raw and processed tadpole meal. The microbes recorded from the lower temperature could have resulted from water crystals in feed, which could lead to rapid microbial spoilage and destruction of moisture-sensitive vitamins like vitamin C (Wilson *et al.*, 2009) due to of moisture and low temperature and high humidity. Decrease in Vitamin E, which could result from high rancidity of feed, has been associated with mould appearance in meals because of fungal destruction of α – tocopherols (Falaye, 2006). Some of the inconsistent trends in microbial growth and nutrient depression in feed can possibly be due to the interactions taking place between chemicals present in the feed, processing methods, preservation technique and resistance of the organisms as reported by Daly (2005). The fact that microbial growth was reported all diets studied despite their compositions and place of storage corroborate with the report of Fagbenro *et al.* (2008) that no feed was completely free of fungi contamination. The occurrence of Aspergillus is significant in public health. A niger and A. flavus had been reported (Houlihan *et al.*, 2001) as the common agents of food spoilage most especially in the tropics where their spores are widely distributed. Some species are known to secrete toxins known as aflatoxin which cause food poisoning and are carcinogenic to man; when ingested affect the liver and no effective therapeutic treatment has yet been known. Aspergillus spp caused "Aspergillosis" (a disease of the lungs) (Robert, 2009). Many human and animal diseases such as mycotic abortion, aflatoxin poisoning, allergic reaction, systemic infections are attributed to mould and fungi ingestion

(Sogbesan, 2006). Penicillum spp and Fusarium spp are also capable of secreting toxins like ichra toxins and penicillic acid that are dangerous to human health. Various lung diseases in farmers are associated with mould and grain dust. Aflatoxins, even at diminutive dietary levels have been established to decrease growth rate and feed conversion efficiency (Ajah, 2005) in animals fed such feed.

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Appendix 1

Proximate composition of tadpole meal

					95% Confidence Interval for Mean			
	N	**Mean**	**Std. Deviation**	**Std. Error**	**Lower Bound**	**Upper Bound**	**Minimum**	**Maximum**
5	4	12.2225	.93179	.46589	10.7398	13.7052	10.83	12.80
6	4	16.0950	.97695	.48848	14.5405	17.6495	14.75	16.88
7	4	18.5350	1.39204	.69602	16.3200	20.7500	16.93	20.18
8	4	21.8150	2.23978	1.11989	18.2510	25.3790	19.90	24.78
9	4	22.9325	1.73654	.86827	20.1693	25.6957	21.60	25.45
10	4	28.2225	3.23479	1.61740	23.0752	33.3698	24.60	32.28
11	4	31.0950	4.70281	2.35141	23.6118	38.5782	27.40	37.73
12	4	33.8925	5.25537	2.62768	25.5300	42.2550	30.00	41.60
Total	32	23.1012	7.62458	1.34785	20.3523	25.8502	10.83	41.60

ANOVA

VAR00001

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1586.186	7	226.598	25.180	0.000
Within Groups	215.975	24	8.999		
Total	1802.162	31			

The ANOVA table reports a p-value (0.00) which is less than 0.05 which signifies that at least one pair of treatment level has a significant difference in weeks, hence the Duncan multiple range tests is used to show the pair of means which are significant.

Appendix 2

Growth indicies of claris gariepinues fingerlings for whole tadpole meal diets for 70days.

VAR00001	N	Mean	Std Deviation	Std Error	Lower bound	Upper Bound	Minimum	Maximum
5	4	13.6825	2.15636	1.07818	10.2512	17.1138	11.40	16.48
6	4	17.8775	2.98112	1.49056	13.1339	22.6211	14.88	21.38
7	4	20.3500	2.88617	1.44309	15.7575	24.9425	18.00	24.40
8	4	24.7775	3.99613	1.99807	18.4188	31.1362	19.90	28.28
9	4	28.8700	5.68512	2.84256	19.8237	37.9163	22.00	35.30
10	4	32.9775	7.63311	3.81656	20.8315	45.1235	24.60	42.90
11	4	35.5300	7.62801	3.81400	23.3921	47.6679	27.04	45.45
12	4	38.3625	7.91153	3.95576	25.7735	50.9515	30.00	49.00
Total	32	26.5534	9.74605	1.72287	23.0396	30.0673	11.40	49.00

ANOVA

VAR00001

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2196.949	7	313.850	10.075	0.000
Within Groups	747.600	24	31.150		
Total	2944.548	31			

The ANOVA table reports a p-value (0.00) which is less than 0.05 which signifies that at least one pair of treatment level has a significant difference in weeks, hence the Duncan multiple range tests is used to show the pair of means which are significant.

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