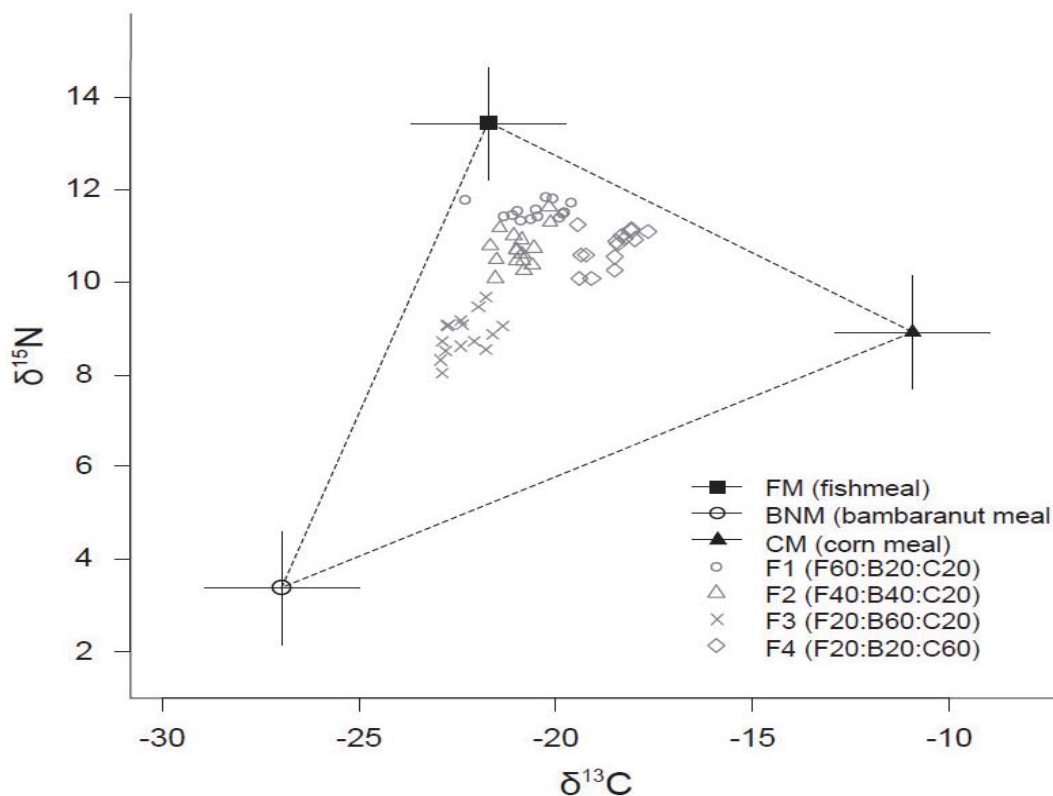


Uchchukwu Dennis Enyidi

Production of Feeds for African Catfish *Clarias gariepinus* Using Plant Proteins



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Using Plant Proteins

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UNIVERSITY OF JYVÄSKYLÄ

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Catfish *Clarias gariepinus*
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UNIVERSITY OF JYVÄSKYLÄ

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ABSTRACT

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Yhteenvedo: Kasviproteiinien käyttö jättikonnamonnin *Clarias gariepinus* rehuissa
Diss.

Aquaculture is the fastest growing agricultural sector. Fishmeal (FM) is the major protein feedstuff in fish feeds but supply is negatively affected by overfishing and excessive demand than supply leading to price increases. For sustainable aquaculture development there is need to reduce the FM content of fish diets. Plant proteins are plausible FM alternatives but they contain anti-nutritional factors and are deficient in some essential amino acids. These shortcomings interfere with the fish health, assimilation and utilization of plant ingredients. It is therefore important to know the ingredients assimilation, utilization and contributions to fish biomass while supplementing FM with plant proteins. Analyses of naturally occurring stable isotopes of ^{13}C and ^{15}N in fish can reveal the assimilation, utilization and biomass contribution and nutrient partitioning of feed ingredients. African catfish *Clarias gariepinus* grows very fast and has been introduced to many countries outside Africa. To this end we supplemented FM with soybean (*Glycine max*) meal (SBM), bambaranut (*Voandzeia subterrenea*) meal (BNM), sesame (*Sesamiun indicum*) seed meal (SSM) or corn (*Zea mays*) meal (CM) in novel diets for African catfish. The C and N stable isotope signatures of the ingredients and fish were analyzed. Simple stable isotope linear mixing model and model stable isotope analyses in R (SIAR) were used in analyzing catfish assimilation of the feeds. Results showed that in larval African catfish diets, BNM cannot be included beyond 25 % as substitute of FM or SBM without compromising growth. However, BNM can completely replace SBM in the diets of fingerling African catfish without negative growth effects. SSM is a good substitute of FM and can replace up to 60 % of FM in catfish diets. Also SSM in combination with BNM is good supplement of FM, producing very fast growth. CM was poorly utilized by the catfish but could serve as basal ingredient.

Keywords: African catfish, assimilation, bambaranut; sesame seed; soybean; specific growth rate; stable isotopes.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers which will be referred to in the text by their Roman numerals I-IV.

Article I; I developed the research idea and consulted with my supervisors and co authors. Together with my supervisors J. Pirhonen and J. Vielma and co-authors we planned out the research and modalities. I bred the fish and was responsible for carrying out the experiments, data collection and drafting the ms. Co-authors contributed in analyses.

Article II; I planned the research with my supervisors. J. Vielma designed the research, J. Pirhonen was in charge of daily supervision and I was responsible for day to day carrying out of the experiment. I also was responsible for data analyses and drafting the ms.

Article III; I developed the research idea with my supervisors. J. Pirhonen was in charge of daily supervision. I was responsible for carrying out of the research, data collection, isotope analyses and drafting the ms.

Article IV; I developed the research idea and in conjunction with my supervisors planned the research. The mixing methodology was designed by J. Kettunen. I was responsible for carrying out the experiment, data collection and isotope analyses. I was also responsible for drafting the ms.

- I Enyidi U., Kiljunen M., Jones R.I, Vielma J. & Pirhonen J. 2012. Nutrient assimilation by first-feeding African catfish (*Clarias gariepinus*) assessed using stable isotope analysis. Accepted: *Journal of the World Aquaculture Society*.
- II Enyidi U., Pirhonen J. & Vielma J. 2012. Effects of substituting soybean meal with bambaranut (*Voandzeia subterranea*) meal on growth performance and survival of African catfish (*Clarias gariepinus*) larvae. Submitted manuscript.
- III Enyidi U., Pirhonen J. & Vielma J. 2012. Effects of sesame seed meal and bambaranut meal on growth, feed utilization and body composition of juvenile African catfish (*Clarias gariepinus*). Submitted manuscript.
- IV Enyidi U., Pirhonen J., Kiljunen M., Kettunen J. & Vielma J. 2012. Effects of fishmeal replacement with bambaranut meal and soybean meal on African catfish (*Clarias gariepinus*) (Burchell 1822) and assessment of nutrient assimilation using stable isotopes. Submitted manuscript.

1 INTRODUCTION

Aquaculture with 8–10 % annual growth rate is the fastest growing agricultural sector (Anon. 2012). More than half of world food fish are produced through aquaculture (Anon. 2010), which in turn is heavily dependent on aquafeed input (Anon 2012). Aquafeed production must be able to sustain growing world fish demand. Feed production is dependent on a number of protein and energy ingredient sources like fishmeal (FM), fish oil and soybean (*Glycine max*) meal (SBM) which has become costly in international markets (Naylor et al. 2009, Hardy 2010). FM is an excellent protein source for fish feed and it provides essential amino acids and omega 3 and 6 fatty acids (Lech & Reigh 2012). However, the supply of FM is subject to overfishing, environmental catastrophes like earth quakes and also pollution and extreme climatic oscillations such as El Niño (Hardy 2010). Aquafeeds consume about 68 % of total world FM production (Naylor et al. 2009). The constant growth of aquaculture cannot be sustained by dependence on FM as major protein source (Hardy 2010). There is therefore need to reduce aquaculture dependence on fishmeal and also exorbitant plant proteins like soybean meal.

The reduction of FM could increase profitability of fish farming, reduce fishing pressure on species used in FM production and reduce organic load of fish farm effluents and its environmental pollution (Naylor et al. 2009). Plausible alternatives to FM are ingredients of animal by-products origin like poultry by-products (Goda et al. 2007), combined poultry by-products and meat and bone meal (Hu et al. 2008, Wang et al. 2008), feather meal and blood meal (Wang et al. 2008), fish-offal (Mondal et al. 2008) and other local fisheries by-products such as crab meal (García-Ortega et al. 2010). A major problem with animal by-products is health hazards due to disease outbreak like birds flu, swine fever virus and mad cow diseases. Animal by-products are also subject to government regulations. Plant proteins are abundant (Gatlin et al. 2007) and on the other hand economical and have less dioxins and PCB's than FM (Tacon & Metian 2008). This may not be exactly applicable in areas of Northern hemisphere with persistent organic pollutants that are of human health concern. The global pesticide use shows that North America uses 27 %, Western

Europe 24 %, Latin America 14 %, Asia 25 % and rest of the world 10 %. Similarly herbicide use record shows that North America uses 35 %, Western Europe 30 %, Japan and Australia 15 % and rest of the world 20 % (Pacanoski 2007). However due to low industrialization (with exception of oil producing areas) and persistent traditional agriculture practices, plant proteins from areas like Africa, South America and parts of Asia have little or no organic pollutants.

Soybean is presently the most used plant protein in fish feed production and also for African catfish *Clarias gariepinus* (Shipton & Hetch 2005). Plant proteins like sunflower (*Helianthus annuus*) seed cake, bean (*Phaseolus vulgaris*) meal (Nyina-Wamwiza et al. 2007), cotton (*Gossypium* sp.) seed cake (Imorou Toko et al. 2008), and pea (*Pisum sativum*) seed meal (Davies & Gouveia 2008) and groundnut (*Arachis hypogaea*) cake (Davies & Ezenwa 2010) have been analyzed as possible replacements for FM in diets of African catfish. Plant protein supplements with potential for application in fish feed production must also be easily available and cost efficient (Gatlin et al. 2007). Furthermore, plant protein substitutes of fishmeal must be nutritionally balanced, environmentally friendly and preferably also beneficial for human health (Gatlin et al. 2007).

High-protein soybean meal contains around 48 % crude protein while soy protein concentrate contains around 65 % crude protein (Gatlin et al. 2007, Salze et al. 2010). Soybean has good content of amino acids although it is poor in sulphur amino acids like methionine (Cai & Burtle 1996, Gatlin et al. 2007). The SBM is also deficient in lysine and threonine (Gatlin et al. 2007). Although soy protein concentrate is very high in protein its use is restricted by high price (Gatlin et al. 2007). Soy protein concentrate is also used for human food making it even less attractive as aquafeed ingredient. Multi usages like as human food item, food additive and animal feed item, vegetable oil production, and as biofuel have made soybean costly (Hill et al. 2006, Hardy 2010). Hence, soybean as a multipurpose raw material is competitively scarce and expensive for aquaculture in sub-Saharan African (Shipton & Hecht 2005, Ayinla 2007, Azaza et al. 2009).

Consequently, depending on the region, there may be need to seek alternatives to both fishmeal and soybean meal. This thesis examined the use of the following alternatives; soybean meal (SBM), bambaranut (*Voandzeia subterranea*) meal (BNM), sesame (*Sesamum indicum*) seed meal (SSM) and corn (*Zea mays*) meal (CM) as fishmeal or soybean supplements.

Bambaranut is a proteinous legume while sesame seeds are high in protein and oil and both plants have untapped potentials in fish feed industry especially in sub-Saharan Africa and Asia. Bambaranut has been shown to have high amount of amino acids like lysine and methionine (Dakora & Muofhe 1995), while sesame seeds are high in methionine and has also other sulphur amino acids (Lee et al. 2003, Hahm et al. 2009). Sesame seeds are good source of C₁₈ group essential fatty acids (linoleic and linolenic acids) (Nzikou et al. 2009).

In some experiments in this thesis fishmeal was partially substituted with SBM and BNM. SBM was also substituted with BNM and both completely replaced FM in (IV). In another experiment BNM was also substituted with SSM

and the effects of combining SSM with BNM as FM replacements were tested. FM was substituted with CM and BNM for first feeding African catfish.

1.1 Substituting fishmeal with plant proteins

Substitution of fishmeal with plant proteins has been reported to reduce growth rate especially of carnivorous and omnivorous species (Krogdahl et al. 2003, Hansen et al. 2007). Inclusion of plant ingredients like sunflower (*Helianthus annuus*) cake (Nyina-Wamwiza et al. 2007), cotton (*Gossypium* sp.) seed meal (Imorou-Toko et al. 2008), soybean, corn, wheat (*Triticum aestivum*) middling's and cotton seed meal (Li et al. 2010) in fish feed have been reported to reduce fish growth rate in comparison to fishmeal. Similarly plant ingredient like leaf meal of *Amaranthus spinosus* (Adewolu & Adamson 2011), was found to reduce fish growth rate compared to fishmeal diets. The reduced growth rate of fish fed with plant proteins is due to several reasons such as anti-nutritional factors (ANFs) inherent in most plant ingredients (Francis et al. 2001), imbalanced amino acid composition (Xie et al. 2001) or poor digestibility (Lech & Reigh 2012). The replacement of fishmeal with plant proteins may also reduce feed palatability (Nyina-Wamwiza et al. 2007, Tiril et al. 2008) and consequently reduce feed intake (Peres et al. 2003, Nyina-Wamwiza et al. 2007, Tiril et al. 2008).

Some of the ANFs present in plant ingredients are phytic acid, fibers, oligosaccharides, enzyme inhibitors, saponin and glucosinolates (Francis et al. 2001). ANFs reduce fish growth and interfere with health and wellbeing of the fish (Francis et al. 2001, Gatlin et al. 2007). Some thermo labile ANFs like protease inhibitors in legumes can be inactivated or reduced by heating, but over heating may reduce protein quality (Krogdahl & Holm 1983, Gatlin et al. 2007). Phytic acid is heat stable and could be reduced by application of exogenous enzyme like phytase (Vielma et al. 2004).

1.1.1 Bambaranut

Bambaranut is herbaceous legume from the family Fabaceae and is of African origin (Obizoba & Egbuna 1992, Basu et al. 2007). Bambaranut is grown all over Africa primarily for human consumption (Obizoba & Egbuna 1992, Goli 1995). Bambaranut meal is made from bambaranut after removal of bambaranut sievates (Onyimonyi & Ugwu 2007). Bambaranut has high amount of essential amino acids lysine, cystine and methionine (Dakora & Muofhe 1995). The crude protein content of bambaranut is 24–28 % (Obizoba and Egbuna 1992, Dakora & Muofhe 1995, Basu et al. 2007), and the lipid content is 12–18 %. Bambaranut lipids feature majorly linolenic, linoleic, palmitic acids and stearic acids (Minka & Bruneteau 2000). BNM has ANFs like polyphenols and trypsin inhibitors (Poulter 1981). Bambaranut has over 50 % carbohydrates (Sirivongpaisal 2008) and is estimated to contain about 30 % neutral sugars identified as glucose and

galactose (Minka & Bruneteau 2000). There are also oligosaccharides in the meal and bambaranut meal has very high oil absorbance of $1.30 \pm 0.06 \text{ ml g}^{-1}$ (Sirivongpaisal 2008). This oil absorbance can be crucial factor in the usages of bambaranut meal. Bambaranut is cheap and abundant but classified as neglected crop meal. It is produced abundantly in sub-Saharan Africa but is not in high demand as human food (Hillocks et al. 2012). Nutritional value and abundance makes BNM a good candidate for supplementing fishmeal in aquafeeds. The BNM used in this thesis was made from white bambaranut cultivar and imported from Enugu, Nigeria.

1.1.2 Corn

Corn is popular cereal crop of the family Poaceae. Corn meal (CM) is used in aquafeeds mainly as basal ingredient together with other major ingredients. CM has very low amount of all essential amino acids. However, CM is a good source of carbohydrates and African catfish is known to utilize carbohydrates very well (Ali & Jauncey 2004). Corn gluten meal, a product with elevated protein content compared to CM, has rather been used in supplementing fishmeal and soybean in aquafeeds for rainbow trout (*Oncorhynchus mykiss*) (Morales et al. 1994), sea bream (*Sparus aurata*) (Robaina et al. 1997) and for Nile tilapia (*Oreochromis niloticus*) (El-Biary 2005). CM is mainly used as extra additive in diets of herbivorous fish like *Tilapia zilli* (Adewolu 2008). The corn meal used in this experiment was made from corn imported from Enugu, Nigeria.

1.1.3 Sesame

Sesame (family Pedaliaceae) is grown all over tropical and sub-tropical world (Sen & Bhattacharyya 2001, El-Adawy & Monsour 2011). Sesame seeds are source of essential amino acids and sulphur amino acids like methionine (Lee et al. 2003, Hahm et al. 2009). Furthermore, sesame seeds contain sesamin, sesamol, sesaminol and sesamol (Sirato-Yasumoto et al. 2001, Jacklin et al. 2003, Chang et al. 2010). Sesame seeds have about 41–58 % oil, 18–25 % protein and 13–17 % carbohydrates (Bahkali et al. 1998, Kang et al. 2003, Yusuf et al. 2008). Sesame seed meal (SSM) has been shown to replace up to 16 % of soybean meal (SBM) in diets of Nile tilapia without negative growth effects (Guo et al. 2011). Sesame cake has also been shown to replace 50 % of FM in the diets of juvenile rainbow trout without amino acid supplementation (Nang Thu et al. 2011). However, inclusion of sesame seed meal has been noted to cause reduced feed intake in carp (*Cyprinus carpio*) (Hasan et al. 1997), rohu (*Labeo rohita*) (Mukhopadhyay & Ray 1999) and Indian carp (*Cirrhinus mrigla*) (Singh et al. 2003). Sesame seeds contain high levels of oxalic acid especially within the hull which impart bitter taste (Carbonell-Barrachina et al. 2009). Tannin and phytic acids have also been reported in sesame seed meal (Mukhopadhyay & Ray 1999). Furthermore, SSM has low content of lysine but supplementing sesame diets with lysine improved growth and feed utilization in *Labeo rohita*

(Mukhopadhyay & Ray 1999). The sesame seeds that were used in this thesis were unshelled and obtained from a supermarket in Finland.

1.1.4 Soybean

Soybean meal (SBM) is currently the major plant ingredient used in fish feed production (Barros et al. 2002, Drew et al. 2007). Soybean meal is also the primary plant protein source in African catfish diets (Shipton & Hecht 2005). Soybean has ANFs like protease inhibitors, lectins, phytic acids, saponin, phytoestrogens and allergens (Francis et al. 2001). Inclusion of SBM above 50 % in diets of African catfish caused reduced feed utilization and growth compared to fishmeal (Fagbenro & Davies 2001, Imorou Toko et al. 2008). Similarly, reduced feed intake due to inclusion of soybean meal has been noted in channel catfish (*Ictalurus punctatus*) (Peres et al. 2003) and African bonytongue (*Heterotis niloticus*) (Monentcham et al. 2010). Even though soybean has good amino acid profile it is deficient in essential sulphur amino acids (Gatlin et al. 2007). Phytic acid can be reduced in soybean based diets by top spraying the diets with phytase enzyme, or by phytase pre-treatment of feed ingredients, to improve digestibility of dietary phosphorus (Vielma et al. 2004). Soybean meal used in this thesis research was obtained from a supplier in Finland.

1.1.5 Ingredients used in substituting fishmeal in this thesis

The plant ingredients utilised in substituting fishmeal in this thesis were as follows: bambaranut meal (BNM), sourced from Enugu Nigeria. The crude protein was 21 %, lipid 8 %. Sesame seed (SSM) was sourced from supermarket in Finland and the crude protein was 18.1 % and the lipid content was 40.5 %. The corn meal used in the experiment was sourced from Enugu Nigeria and the crude protein content was 8 % and crude lipid content was 2 %. Soybean meal (SBM) was deffated and obtained from Finland and the crude protein content was 42 % and the crude lipid content was 11 %. The fishmeal used in the experiment was obtained from supplier in Finland and crude protein was 70 % and crude lipid 10-12 %.

1.2 African catfish *Clarias gariepinus*

African catfish is one of the best aquaculture species under culture (Anon. 2006). The aquaculture potential of African catfish is enhanced by the following qualities it possesses, (i) ability to feed on almost anything, (ii) high fecundity, (iii) ability to survive harsh pond rearing conditions, (iv) fast growth and disease resistance, and (v) ability to survive outside water using atmospheric oxygen (De Graaf & Janssen 1996, Ali & Jauncey 2005). Consequently, African catfish has been introduced into many countries like the Netherlands, Hungary,

Denmark, Germany, Spain, England, Belgium, USA, most Asian countries and South America (De Graaf & Janssen 1996, Anon. 1997).

Nigeria is the leading catfish fish producer with 100 000 t in sub Saharan Africa (Anon. 2012). There is production forecast of 130 000 t by the year 2015 (Shipton & Hetch 2005). Despite increased production, profitability of culture can be very low. The major cause of low profitability is high cost of imported feed, and high cost of fishmeal (Hecht 2005). In Nigeria 30 % of fish feed used is commercially manufactured (i.e. nutritionally balanced but expensive as an imported item) while the rest, appr. 70 %, is farm made (i.e. likely nutritionally imbalanced but relatively inexpensive) (Hecht 2005).

1.3 Assimilation of nutrients and stable isotope analyses

There are basically three major types of plants based on their photosynthetic biochemistry. These are the C₃, C₄ and CAM (Crassulacean acid metabolism) plants. The C₃ plants attach photosynthetic carbon dioxide by ribulose-bisphosphate to form G3P (glyceraldehyde-3-phosphate). G3P is structurally a 3-carbon compound which is its intermediate for glycolysis hence it is named a C₃ plant. The C₄ plants forms oxaloacetate by fixing carbon dioxide by phosphoenolpyruvate carboxylate (PEPC) in the mesophyll cells (Tiunov 2007). Oxaloacetate is structurally a 4-carbon compound and also functions as intermediate for glycolysis, hence it is called a C₄ plant. Most temperate areas have C₃ plants and the tropical areas C₄ plants. The CAM plants are mainly arid plants like cactuses, agaves and make better use of water. This classification becomes important in the stable isotope signature analyses when plant proteins are utilized. The carbon classifications of the plant proteins are important to avoid overlap and in determining the separation of the isotope signatures during stable isotope analyses. In this thesis bambaranut, soybean and sesame seed meals were utilized. Bambaranut is known to be of tropical origin and hence should be a C₄ plant, soybean and sesame seed are C₃ plants.

The knowledge on recipient fish assimilation of plant proteins and the fishmeal they substitute is important in feed formulation. It is also important to know nutrient partitioning (fish separation of component dietary nutrients) from compounded diets (Beltràn et al. 2009). Knowledge of nutrient routing and assimilation are essential for optimization of feed mixes. Feed ingredient utilization and digestibility in aquaculture are traditionally determined by digestibility tests either by using inert chemical markers such as chromium oxide or yttrium oxide (Glencross et al. 2003, Oliveira et al. 2008, Gaylord et al. 2008) or by radioactive ¹⁴C (Hovde et al. 2005). Radioactive markers have also been used for measuring nutrient digestibility and assimilation in fish larvae (Conceição et al. 2001, Izquierdo et al. 2001, Morais et al. 2006). However, the use of radioactive substances for digestion and assimilation studies poses risks to users, and therefore their use is subjected to regulations (Preston et al. 1996, Schleichriem et al. 2004).

Ratios of naturally occurring stable isotopes of carbon ($^{12}\text{C}/^{13}\text{C}$) and nitrogen ($^{14}\text{N}/^{15}\text{N}$), usually expressed as $\delta^{13}\text{C}$ - and $\delta^{15}\text{N}$ -values, could provide an alternative method of measuring assimilation and retention in fish nutritional studies. This method has been used during the last decade in ecological studies (Fry 2006, Dubois et al. 2007, Yokoyama et al. 2009, Redmond et al. 2010), and fish larval nutritional studies (Schlechtriem et al. 2004, Jomri et al. 2008). Feed ingredients used in compounding diets usually have variable and different isotopic signatures (Post 2002, Beltràn et al. 2009, Redmond et al. 2010). Variable isotope signatures of the ingredients enable analyses of routing and assimilation using stable isotope ratios of carbon and nitrogen (Schlechtriem et al. 2004, Jomri et al. 2008, Beltràn et al. 2009).

In animals, stable isotope ratio of carbon and nitrogen reflect their food's isotopic ratios plus a small fractionation or trophic shift (DeNiro & Epstein 1981). The lighter isotopes (^{12}C and ^{14}N) are more readily used in body metabolism (DeNiro & Epstein 1981, Schoeller 1999, Fry 2006), making the body enriched with heavier isotopes (^{13}C and ^{15}N) (DeNiro & Epstein 1981, Olive et al. 2003, Fry 2006, Dubois et al. 2007).

Similarly growth rate or tissue turnover rate are important in reflecting isotopic values of fish diets (Sakano et al. 2005). Tissue turnover rate is equal to growth rate for very fast growing fish but vary among organs and tissues like liver, bone and muscle (Bosley et al. 2002, Guelinckx et al. 2007). Fast growing larvae can have a total tissue turnover within days (Herzka & Holt 2000).

Larval African catfish possesses higher growth rate and food conversion efficiency when compared to most other fishes (Keckis & Schiemer 1992, Conceição et al. 1998). The analyses of incorporated dietary isotopes using simple linear mixing and mass models show contribution of different feed ingredients to fish biomass (Kwak & Zedler 1997, Phillips 2001, Burford et al. 2004). Incorporated dietary isotopes have also been analyzed using Euclidean distance (distance between the isotope signature of consumer and the feed) model (Szepanski et al. 1999). Linear mixing models can be used to partition double source diets using single stable isotope signature like $\delta^{13}\text{C}$ or triple source using additional isotope signature of $\delta^{15}\text{N}$. There has been much improvement in estimation of diet source partitioning using linear mixing model (Phillips 2001, Philips & Greg 2001, Lubetkin & Simenstad 2004, Moore & Semmens 2008, Parnell et al. 2010, Ward et al. 2011).

1.4 Experimental scope and study aims

This thesis examines effects of formulating novel feeds for African catfish using cheap plant proteins either as supplements of fishmeal, as combined with other plant proteins at constant fishmeal levels or as supplements to other plant proteins. The thesis also examines assimilation of nutrients from the diets and the ingredients contributions to fish biomass.

The assimilation of the nutrients and biomass contribution was examined using stable isotopes of C and N. This is in order to elucidate the assimilation and biomass contribution of major protein sources in the diet. The feeds were made and tested on three different developmental life stages of the African catfish. The catfish has five main stages of development. The first stage is yolk sac larvae feeding exclusively on the yolk sac. Yolk sac larvae develop to first feeding larvae after 4–5 days depending on water temperature (African catfish needs between 26–30 °C for optimal development and external feeding can begin after 50h at 28 °C–30 °C) (Janssen 1987). The first feeding African catfish mainly feeds on live diets like *Artemia* for the first five days after which they can be weaned onto dry diets. The first feeding larvae develop to advanced post larvae which eventually develop to the fingerling stage. The fingerlings subsequently develop to become the adults. Developmental stages are stated as below:

- a) Yolk sac to first feeding larvae
- b) First feeding larvae to post larvae
- c) Post larvae to fingerling
- d) Fingerlings to juvenile
- e) Juvenile to adult

To this end novel feed were formulated and tested for the catfish in the following stages;

- (a) The first feeding to post larvae (I): Fishmeal was supplemented by bambaranut meal and corn meal. Assimilation of nutrient from ingredients and their biomass contributions to larval biomass was analyzed using the model stable isotope analyses in R (SIAR). The observed and expected contributions of nitrogen to fish biomass from the feed were analyzed using simple regression analysis. Leave out-one cross validation was used in validating relationship of the observed and expected values plot.
- (b) Post larvae to fingerling (II): Bambaranut meal substituted soybean in the diets of post larval African catfish. The growth and nutritional performances of the catfish and dietary amino acid contents were analyzed. Simple regression analysis was used in analyzing effects of substitution on growth and nutritional parameters.
- (c) Fingerlings to juvenile (III): Bambaranut meal was substituted with sesame seed meal. Bambaranut meal and sesame were both individually used together with fishmeal in producing novel diets for the catfish. The assimilation of nutrient and their biomass contribution was analyzed for the two protein source diets with stable isotope linear mixing model. The assimilation from three protein source diets was analyzed with the model stable isotope analyses in R (SIAR).
- (d) Fingerlings to juvenile (IV): Bambaranut meal and soybean meal were used in supplementing fishmeal in diets of the catfish. Bambaranut meal was also used together with soybean meal and fishmeal in diets for the African catfish. The assimilation from the protein sources in the diets was analyzed using stable isotope analyses in R (SIAR).

2 MATERIAL AND METHODS

2.1 Stocking density

The stocking density of the catfish was first examined to determine the density effects on specific growth rate, survival, food conversion ratio and the weight gain of catfish. Therefore, catfish (average initial weight \pm SD) 1.71 ± 0.26 g were stocked at low density (9 fish per glass aquarium of width 24 cm, height (water level) 15cm and length 39.5 cm) and at high density (90 fish per aquarium). The fish were fed rainbow trout diet for 42 days. The fish stocked at low density grew at specific growth rate of approx. $1 \% \text{ day}^{-1}$ while those at high density grew at approx $5 \% \text{ day}^{-1}$. The fish stocked at high density increased in weight between 3–4 times their initial weights while those at low density did not double their initial weight. The result of the stocking density experiment was a guide in this thesis to ensure welfare, proper growth and nutritional response of the catfish.

2.2 Experimental fish

The first batch of 1000 African catfish fingerlings used in this project was imported from a commercial fish farm in the Netherlands. Thereafter experimental fish were produced by artificial fertilization of African catfish brooders (grown from the imported fingerlings) maintained in the wet laboratory of the Department of Biological and Environmental Science, University of Jyväskylä. The female brooder was maintained in a flow-through tank at $28\text{ }^{\circ}\text{C}$ after a GnRH α hormone (Ovopel) injection. Ovopel contains metoclopramide, a blocker of dopamine receptors, and mammalian hormone GnRH analogue (D-Ala $_6$, Pro $_9$ NET-mGnRH) (Horváth et al. 1997). Ovulation, stripping and fertilization were completed after 8 h. The eggs were incubated in 15 l flow-through glass aquaria at $28\text{ }^{\circ}\text{C}$ and the eggs hatched after c. 24 h post

fertilization. The hatchlings were reared in 15 l flow through aquaria till used for the experiments. The physico-chemical parameters of the aquarium water per experiment are documented in the articles I-V.

2.3 Diet formulation, feeding and experimental set up

2.3.1 First feeding to post larvae (I)

The experimental diets were produced by substituting fishmeal (FM) with bambaranut meal (BNM) and corn meal (CM). The percentage (%) inclusion levels of FM:BNM:CM ranged from 20 % to 60 %. In addition, three more diets (FM, BNM and CM) were produced based entirely on the individual ingredient (Table 1). There was no vitamin or mineral premix added to the diets since the research was to examine the growth and assimilation of the ingredients in their raw form. Experimental diets were labelled as feed 1 (F1) to feed 4 (F4).

Appropriate levels of the ingredients were measured according needed quantity in composition and mixed (Table 1). Approximately 0.2 l of water was added to the mixture and stirred with electric mixer. There was no fish oil added to mixture. After the mixing, samples of each feed type was taken and placed in glass vials and stored at -80°C until stable isotope analyses. Mixed ingredients were preconditioned with an electric cooker at 100°C . Preconditioned dough was pelleted using a kitchen meat mincer and pellets were oven dried at 70°C for 18 h. After diet processing, 50 g feed samples per diet were weighed and placed in plastic cellophane sachets for proximate analyses and in glass vials for stable isotope analyses and stored at -80°C . The dried pellets were ground to dust and packed per feed type in airtight plastic containers and stored at -20°C till used in feeding. The catfish were stocked in three replicate aquaria and hand fed *ad libitum* four times daily for 30 d with the experimental diets. Precautions were taken to avoid over wastage or escape of food particles with outflow water.

2.3.2 Post larvae to fingerling (II)

The experimental diet used in this experiment was made by varying inclusions of soybean meal (SBM) and bambaranut meal (BNM) with constant amount of fishmeal (FM). The plant proteins constituted 50 % of the FM content of the diets. Five experimental diets, feed 1 (F1) to feed 5 (F5), were produced to vary in percentage inclusion of (SBM) and (BNM) (Table 2). In addition, all feeds contained 60 % of fish meal, 8 % of wheat flour and 2 % vitamin premix. All ingredients were mixed and 0.5 l of water was added and the dough was steamed and preconditioned for 30 min using an electric cooker. The dough was pelleted using a meat mincer and pellets were dried in an oven at 70°C . Dried feed was ground to powder and stored in air tight bags and stored at -20°C till used. African catfish of initial weight (average \pm SD, n= 20) 0.15 ± 0.02 g

were stocked at 350 larvae per two replicate aquaria per feed type. Two replicates were considered adequate due to the regression design of the trial. The fish were fed to apparent satiation three times daily (0800–0900, 1200–1300, 1800–1900).

TABLE 1 Composition and proximate values of diets used in feeding first-feeding African catfish larvae for 30 days. Ingredient levels for compounding feeds (F1–F4) are in percentages (%). The single ingredients fishmeal (FM), bambaranut meal (BNM) and corn meal (CM) were also fed to fish separately (I).

Ingredients	F1	F2	F3	F4	FM	BNM	CM
Bambaranut meal	20	40	60	20		100	
Corn meal	20	20	20	60			100
Fishmeal	60	40	20	20	100		
Total	100	100	100	100	100	100	100
Proximate composition							
Moisture	7.13	6.22	5.61	5.07	6.70	5.50	6.00
Protein	47.80	38.08	28.20	23.00	65.50	21.00	8.00
Lipids	9.50	7.89	6.90	4.30	12.00	8.00	1.98
Ash	6.68	8.43	8.45	8.34	5.89	0.23	1.00

TABLE 2 Composition and proximate values of diets used in feeding larval African catfish larvae for 30 days. Ingredient inclusion levels for compounding feeds (F1–F5) are in percentages (%) and vary in soybean meal (SBM); bambaranut meal (BNM) -ratio (II).

Ingredients	F1	F2	F3	F4	F5
Soybean meal	25	20	15	10	5
Bambaranut meal	5	10	15	20	25
Fishmeal	60	60	60	60	60
Wheat	8	8	8	8	8
Vitamin premix ¹	2	2	2	2	2
Total	100	100	100	100	100
Proximate composition					
Moisture	3.40	3.14	5.22	8.88	8.35
Protein	61.35	61.70	60.35	57.55	56.30
Lipids	14.78	14.01	11.54	11.90	12.57
Ash	5.67	5.24	7.44	8.25	7.19

¹Vitamin premix. The following vitamins (IU kg⁻¹ diet) were added to supply cholecalciferol, 1300; all-race- α -tocopheryl acetate, 140; vitamins (mg kg⁻¹ diet) menadione sodium bisulfite, 12; thiamin HCL, 8; riboflavin, 16; calcium d-pantothenate, 17; biotin, 0.2; folic acid, 5; vitamin B12, 0.02; niacin, 40; pyridoxine HCL, 16; ascorbic acid (Stay C), 80. The minerals (mg kg⁻¹ diet) added were as follows: magnesium phosphate, 5000, potassium carbonate, 400, manganous sulfate, 10; ferrous sulfate, 5; zinc sulfate, 80.

2.3.3 Fingerlings to juvenile (III)

In this experiment sesame seed meal and bambaranut meal were used together or alone in combination with same inclusion level of fishmeal. The sesame seed meal was from unshelled sesame seeds. Four diets, feed 1 to feed 4 (F1 to F4), were made varying the percentage inclusion of BNM:SSM (Table 3). The amount of basal ingredients and fishmeal was constant for all compounded treatment feeds (Table 3). The ingredients were blended with an electric mixer for 10 min during which 5 % of water and 5 % of fish oil were added. The mixed dough was then extruded at 130–140 °C with a twin-screw Creusot-Loire cooking extruder using a 1 mm die. The extruded feeds were dried overnight in a drying chamber at 40 °C and stored in air tight plastic bags at -20 °C. The feeds were produced at the Finnish Game and Fisheries Research Institute Laukaa Aquaculture Station. Fingerling African catfish of initial average biomass (\pm S.D) 11.7 ± 0.6 g, $n= 320$, were stocked in four replicate aquaria at density of 20 fish tank⁻¹. The fish tanks were cleaned every morning and the period of cleaning was followed by feeding in the morning. The catfish were fed twice daily, at 0800–0900 and 1700–1800, during the light periods (D16:L8). Fish were fed to apparent satiation in the morning and restricted feeding (3 % of estimated body weight) was given in the evening. This was adjusted as the fish grew.

TABLE 3 Composition and proximate values of diets used in feeding fingerling African catfish. Ingredient levels inclusion in the formulation of feeds (F1–F4) are in percentages (%). Feeds were used in the feeding of African catfish juveniles for 28 days (III).

Ingredients	F1	F2	F3	F4
Bambaranut meal	35.0	23.3	11.7	0.0
Sesame seed meal	0.0	11.7	23.3	35.0
Fishmeal ^a	53.0	53.0	53.0	53.0
Wheat	5.0	5.0	5.0	5.0
Vitamin Premix ^b	2.0	2.0	2.0	2.0
Fish oil	5.0	5.0	5.0	5.0
Total	100.0	100.0	100.0	100.0
Proximate composition				
Moisture	7.13	6.22	5.61	5.07
Protein	46.70	49.50	46.10	45.80
Lipids	17.10	23.18	30.21	33.76
Ash	6.68	8.43	8.45	8.34

^aFishmeal was Icelandic low temperature capelin meal, crude protein 70 % and crude lipid 8 % (source Raisio group, Raisio Finland). ^bVitamin and mineral premix was added to supply the following minerals (mg kg⁻¹ diet): zinc,180; manganese, 60, iodine, 4, and vitamins (IU kg⁻¹ diet); retinol acetate, 300; (vitamins mg kg⁻¹ diet) menadione sodium bisulfite,10; thiamine-HCl, 20, riboflavin, 30, calcium d-pantothenate, 90; biotin, 0.3; folic acid, 6; vitamin B12,0.04; niacin, 120; pyridoxine-HCL, 20; ascorbic acid (stay C), 315; inositol, 200.

2.3.4 Fingerlings to juvenile (IV)

Soybean meal and bambaranut meal were both used to supplement fishmeal in the diets of fingerling African catfish. Protein supplements constituted 60 % of our diets and comprised of different mixes of fishmeal (FM), bambaranut meal (BNM) and defatted soybean meal (SBM) (Table 4). A constant level of 40 % basal ingredients comprising wheat meal, blood meal, vitamin and mineral premixes, and fish oil, was maintained for all feeds. Ground ingredients were mixed and blended with warm water totaling 30 % moisture in the feed mash.

The protein content of the diets was not balanced to avoid fillers and interference with ingredients and diets isotopic signatures. The mixed ingredients were extruded with twin-screw Creusot-Loire cooking extruder using a 1 mm die with barrel temperature of 130 °C–140 °C. Extruded feeds were dried overnight in a warm-air drying chamber at 40 °C and then coated with fish oil in a Dinnissen vacuum oil coater, and stored in air-tight bags until used.

The feeds were formulated using mixing methodology principles (Ruohonen et al. 2003, Ruohonen & Kettunen 2004). Groups of 10 African catfish juveniles, average weight 35.15 ± 0.85 g, were introduced to two replicate 15 l glass aquaria that were connected to recirculation system. Since the experiment was designed with mixing methodology principles dual replication was not problematic, since the flow in ingredients amount were considered. The diets formulation produced three types of diets; (a) single protein source diets (F1–F3), (b) double protein sources diets (F4–F6) and (c) three protein sources diets (F7–F10). The fish were fed experimental diets for 28 d.

TABLE 4 Formulation and proximate composition of experimental diets used in the feeding of African catfish for 28 d. (IV).

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
BNM ^a	600	0	0	300	300	0	200	100	100	400
SBM ^b	0	600	0	300	0	300	200	100	400	100
FM ^c	0	0	600	0	300	300	200	400	100	100
Bloodmeal	100	100	100	100	100	100	100	100	100	100
Wheat	205	205	205	205	205	205	205	205	205	205
Vitam premix ^d	25	25	25	25	25	25	25	25	25	25
Fish oil	70	70	70	70	70	70	70	70	70	70
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Proximate composition										
Moisture	5.5	6.5	6.7	6.3	6.9	6.1	6.1	6.3	6.4	6.2
Protein	27.3	47.0	55.4	39.7	43.9	52.4	43.9	52.1	46.8	37.5
Lipids	10.4	13.8	21.9	13.7	18.1	17.2	17.8	18.2	15.4	13.3
Ash (%)	0.2	0.7	5.9	3.0	4.2	2.2	3.7	5.4	1.5	0.7

^a BNM=bambaranut meal, ^b SBM= soybean meal, ^c FM= fishmeal, ^d vitamin premix=vitamin and mineral premix and were added to supply the following (vitamin IU kg⁻¹ diet): cholecalciferol, 1300, all-race- α -tocopheryl acetate, 140; (vitamin mg kg⁻¹ diet) menadione sodium bisulfite, 12; thiamin HCL, 8; riboflavin, 16; calcium d-pantothenate, 17; biotin, 0.2; folic acid, 5; vitamin B12, 0.02, niacin, 40; pyridoxine HCL, 16; ascorbic acid (Stay C), 80. Minerals: (mg kg⁻¹ diet) magnesium phosphate, 5000, potassium carbonate, 400, manganous sulfate, 10; ferrous sulfate, 5; zinc sulfate, 80. F1–F10 represents feed 1–feed 10. Origin of ingredients and compositions are as stated in section 1.1.5 in this thesis.

2.4 Essential amino acids of ingredients

Essential amino acid values of the ingredients used in formulating the diets were analyzed (Table 5). Amino acid contents of the ingredients were measured using methods of the Anon (1998). Total peptides (bound and free) were analyzed with a Waters Finland Mass Trak UPLC (Water Corporation Milford, USA) and the application was UPLC Amino Acid Analysis Solution®. The essential amino acids were higher in FM than in all plant proteins.

TABLE 5 Essential amino acid content of bambaranut meal (BNM), soybean meal (SBM), fishmeal (FM) and sesame seed meal (SSM) that was used in formulating different diets for experiments in this thesis. Essential amino acid (g kg⁻¹ feed) array of the feed per experiment are detailed in each experiment article attached to this thesis (I-IV).

Amino acids	BNM	SBM	FM	SSM
Arg	16.96	36.70	45.40	31.7
His	6.70	12.20	16.50	5.7
Ile	9.41	21.40	31.30	8.9
Leu	17.24	36.30	51.90	15.7
Lys	13.89	30.80	55.70	5.3
Met	3.11	6.80	20.80	5.3
Cys	2.02	7.50	7.40	7.4
Phe	12.20	24.40	27.10	4.4
Tyr	10.49	17.60	22.01	10.4
Thr	7.27	18.90	29.01	8.2
Val	10.57	25.50	43.00	10.9

Amino acid codes, Arg (arginine), His (histidine), Ile (isoleucine), Leu, (leucine), Lys (lysine), Met (methionine), Cys (cystine), Phe (phenylalanine), Tyr (tyrosine), Thr (threonine), Val (valine). Values of amino acids are g kg⁻¹ feed.

2.5 Stable isotope analyses

In the first feeding catfish research (I) the feed ingredient components used in analyzing of stable isotopes were carefully selected to ensure that the isotope signatures were widely separated. This principle was not possible to apply in III and IV due to mixing of various ingredients. Therefore SIAR model was used to to separate the means and analyze the data. This is such that the stable isotopic signatures of the catfish will be well separated in the model biplot. For measuring the isotope signatures of feed and the whole catfish larvae, samples were freeze dried and ground to powder, and a sample of 0.4–0.6 mg was weighed into tin cups (D4057 Elemental micro analysis, UK). Three replicate samples were analysed at the stable isotope laboratory of the University of

Jyväskylä using a Carlo Erba Flash EA1112 elemental analyzer coupled to Thermo Finnigan DELTAplusAdvantage continuous flow stable isotope-ratio mass spectrometer.

The diets made from double protein sources were analyzed using stable isotope simple linear mixing model (Gamboa-Delgado & Le Vay 2009, Phillips 2012). The diets made from multiple protein sources were analyzed using the SIAR model (stable isotope analyses in R) (Parnell et al. 2010, Phillips 2012).

The stable isotope ratio output results were expressed in standard delta (δ) notation as parts per thousands (‰) relative to the international standard of Vienna Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Two replicate internal laboratory standards (pike (*Esox lucius*) muscle) were run after every ten samples. The analysis also yielded % C and % N values and hence also C: N-ratios for samples. If the results of SIAR analyses are correct, the larval stable isotope signatures will fall within perimeters of triangular bi-plot of Fig. 1-3.

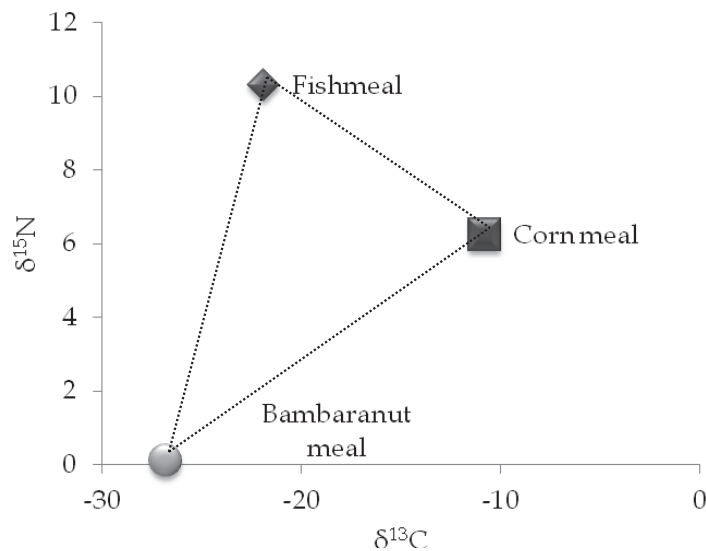


FIGURE 1 The stable isotope signature bi-plot of fishmeal, cornmeal and bambaranut meal ingredients used in compounding diets for first feeding African catfish *Clarias gariepinus* (L).

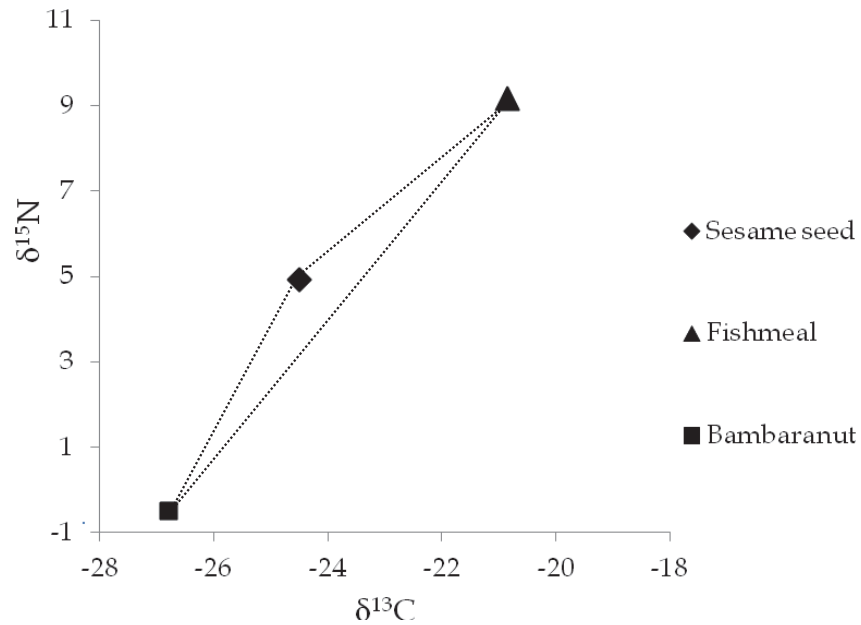


FIGURE 2 The stable isotope signatures bi-plot of fishmeal, sesame seed meal and bambaranut meal ingredients used in compounding diets for fingerling African catfish *Clarias gariepinus* (III).

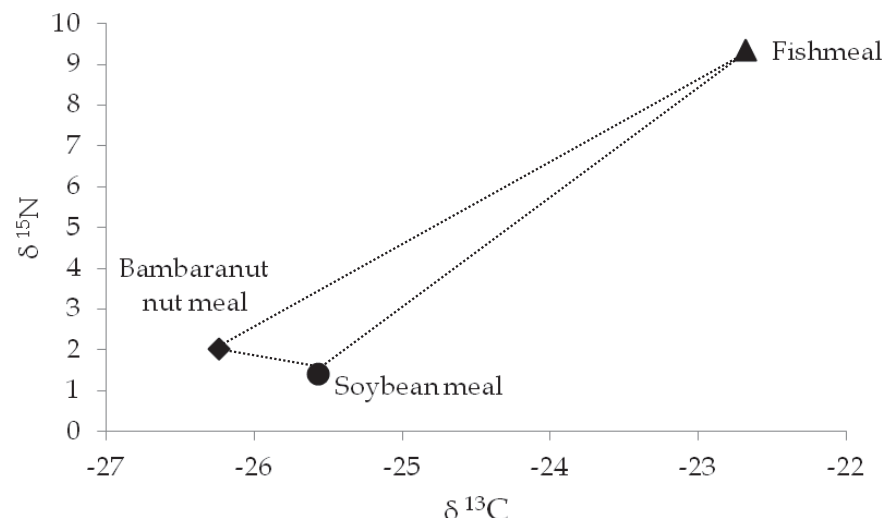


FIGURE 3 The stable isotope signature bi-plot of fishmeal, soybean meal and bambaranut meal ingredients used in compounding diets for fingerling to juvenile African catfish *Clarias gariepinus*. (IV). (Note that the ingredients were processed).

2.5.1 Analyses of stable isotope data

There were two standard runs per sample and two replicate internal standard runs after every ten runs.

The isotope signatures were calculated as follows:

$$\delta X((R_{\text{sample}} / R_{\text{standard}}) - 1) \times 1000$$

where $X = \delta^{13}\text{C}$ or $\delta^{15}\text{N}$

$$\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}}) - 1 \times 1000 \text{ where } R, \text{ is isotope ratio } ^{13}\text{C}/^{12}\text{C}.$$

$$\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}}) - 1 \times 1000 \text{ where } R, \text{ is isotope ratio } ^{15}\text{N}/^{14}\text{N}.$$

2.5.2 SIAR model

SIAR is a package that solves mixing models for stable isotopes using a Bayesian approach. The model is documented in Parnell et al. (2010), Layman et al. (2012) and Phillips (2012). SIAR is sensitive to isotope fractionation and incorporates the isotopic ratio and concentration of nitrogen and carbon (Parnell et al. 2010, Layman et al. 2012). The use of high fractionation values especially when the fish and the diets are not at equilibrium does not offer accurate analysis when SIAR is used. There are some caveats in use of SIAR (Parnell et al. 2010). Most important of them is deviation from normal distribution and the assumption that isotopes are assimilated equally without routing effects. Use of the whole fish for isotope analyses should eliminate problems associated to routing effects. In this thesis whole fish were used in I, II, but also muscle sample was used (IV). The use of muscle instead of whole fish may have added some uncertainty to the results. However, there is evidence of somewhat good representativeness of fish muscle sample when compared to whole fish in isotope analysis (Schielke & Post 2010). The model SIAR had been used in assessing the relative contribution of different food sources to coastal dolphin (Di Benedetto et al. 2011). In general, the isotope methods described in this thesis have been shown to be robust in ecological studies if used properly. The SIAR family of models based on Bayesian statistics has been developed to allow incorporation of uncertainty and variation (such as in nutrient stoichiometry of food sources). In ecological studies particular diet sources can often have considerable isotopic variation, and stoichiometry (e.g. C:N ratios) can vary widely between diets. SIAR allows this kind of variability to be incorporated into the modeling, although of course the more variation there is in the model inputs the greater the range of probabilities there will be in the model outputs.

2.5.3 Fractionation

Isotope fractionation is the ratio of isotope ratios of product for example the consumer, to that of the ingredient (the feed or substrate). The fractionation of the diets in (I) was to be calculated from asymptotic values at equilibrium of fish with diets (Fig. 4). The asymptotic values are the equilibrium isotopic values of the fish and the feed. In order to estimate these values the fish were

analyzed every five days since they were growing very fast. It is expected that isotopic incorporation of dietary signatures would have occurred as the fish accrued more biomass tissue via their specific growth rate. Five individual fishes were taken every five days, and analysed in triplicates (Fig. 4). Otherwise fractionation can be calculated from the stable isotopes values of catfish fed with mixed diets. The calculation of fractionation ($\Delta^{13}\text{C}$ ‰ and $\Delta^{15}\text{N}$ ‰) using mixed diet stable isotopes values was done as follows: from the averages of the values from all mixed diet experiments, as:

$$\Delta^{13}\text{C} / \Delta^{15}\text{N} = \delta K_{\text{consumer}} - \delta K_{\text{diet}}, \text{ where } K = ^{13}\text{C} \text{ or } ^{15}\text{N}.$$

Nitrogen fractionates more than carbon making nitrogen good in defining trophic positions and biomass contribution. Most nitrogen in body system is in form of amino acids (Schoeller 1999). The fractionation of catfish in (IV) was calculated from averages of the catfish fed single protein source diets.

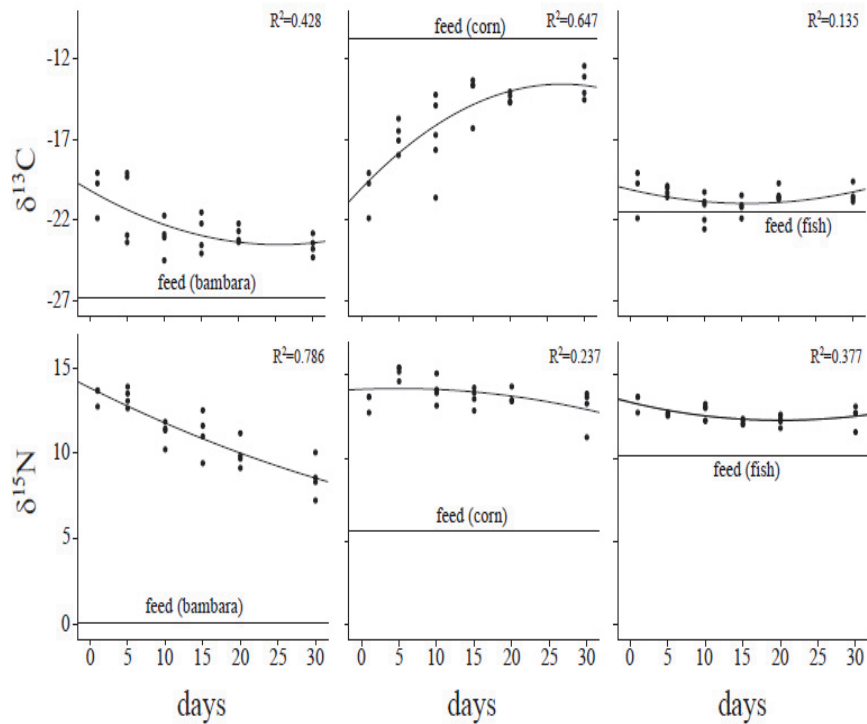


FIGURE 4 Stable isotope signatures of ^{13}C and ^{15}N of five replicate African catfish larvae fed with single diets of fishmeal (FM), bambaranut meal (BNM) or corn meal (CM) for 30 days. Horizontal lines represent isotope values of the feeds (I).

2.6 Calculations and statistical analyses

The nitrogen contribution was estimated by first determining the crude protein of fish. From start of experiment all the protein assimilated by the catfish was assimilated from the diets (fish ate nothing else but the experimental diets). A plot of the observed and expected nitrogen contribution being positively correlated will prove assimilation to be directly proportional to biomass contribution. The specific contribution levels per experiment are documented in I, III and IV.

The sum total of protein contributed by each ingredient per diet, to the catfish crude protein equals the fish crude protein content. This was calculated as follows:

$$\text{Somatic crude protein of fish} = \% \text{ N} * 6.25.$$

Where, %N is from the isotope analyses. 6.25 is a constant multiplier for estimating crude protein from determined sample nitrogen value. All N is assumed to come from fish sample analysed and that is from feed they ate (Levey et al. 2000).

Nitrogen contribution to crude protein per ingredient (%N) calculated as:

$$(\% \text{N}) = (\text{observed N contribution per ingredient} * 6.25).$$

Where observed nitrogen contribution was calculated by multiplying the mean assimilation value of the ingredients (from SIAR) by the %N of the particular diet ingredient and dividing this by total of same calculation for FM, BNM, SBM, SSM or CM as follows:

$$N_{(\text{FM, BNM, SBM, SSM or CM})} = (X_{\text{ass FM}} * \% \text{N of diet}) / (X_{\text{ass FM}} * \% \text{N of diet} + X_{\text{ass BNM}} * \% \text{N of diet} + X_{\text{ass SBM}} * \% \text{N of diet} + X_{\text{ass SSM}} * \% \text{N of diet} + X_{\text{ass CM}} * \% \text{N of diet}),$$

where FM, BNM, SBM, SSM or CM are ingredients of the diets. (Note that diets in equations refer to the feeds, which are made up of the ingredients).

The expected nitrogen contribution is similar to the above but the multiplier $X_{\text{ass (FM, BNM, SBM; SSM or CM)}}$ (i.e. the assimilation of either FM, BNM, SBM, SSM or CM) was changed to $X_{\text{F (FM, BNM, SBM, SSM or CM)}}$ which is the inclusion level of the ingredient (either FM, BNM, SBM, SSM or CM) in the diets.

The values were calculated as follows:

$$N_{(\text{FM; BNM, SBM, SSM or CM})} = (X_{\text{F(FM)}} * \% \text{N of diet}) / (X_{\text{F(FM)}} * \% \text{N of diet} + X_{\text{F(BNM)}} * \% \text{N of diet} + X_{\text{F(SBM)}} * \% \text{N of diet} + X_{\text{F(SSM)}} * \% \text{N of diet} + X_{\text{F(CM)}} * \% \text{N of diet}),$$

where F stand for feed (diet) and $X_{\text{F(FM)}}$ represents the inclusion level of the ingredient (FM, BNM, SBM, SSM, CM) in diet. Note that the diet composition differs according to feed composition in I -IV.

The catfish specific growth rate (SGR, % day⁻¹) = $100 * (\text{Ln } W_2 - \text{Ln } W_1) / t$, where W_1 and W_2 were average weights in g at the start and the end of the experiment and t was the length of the experiment in days.

Food conversion ratio (FCR) was calculated as:

$$\text{FCR} = \text{feed fed (g)} / \text{weight gain (g)}.$$

Condition factor (CF) was calculated as:

$$\text{CF} = 100 * W / L^3,$$

where W is weight of fish in g and L the total length in cm.

Economic conversion ratio (ECR) was calculated as:

$ECR = \text{the price of the variable part in the diet (USD)} * FCR.$

Price of the variable diet fraction was based on the price of the ingredients as given in Fagbenro & Adebayo (2005), Onyimonyi & Ugwu (2007), Obih & Ekenyem (2010). SBM was estimated at 0.42 USD kg⁻¹, BNM 0.07 USD kg⁻¹ and FM 2.23 USD kg⁻¹.

Feed fish equivalence (FFE) was calculated as:

$FFE = 4.5 * FCR * (\% \text{ fishmeal in feed} * 100)^{-1},$

where 4.5 is the ratio of forage fish required to produce 1 kg FM (Boyd et al. 2007).

Protein efficiency ratio (PER) was calculated as:

$PER = FCR * \% \text{ feed protein} * (\% \text{ protein in catfish})^{-1}.$

Protein productive value (PPV) was calculated as:

$PPV = 100 * (\text{final protein content} - \text{initial protein content}) * (\text{protein consumed})^{-1}.$

Daily feed intake (DFI) was calculated as:

$DFI = \% \text{ of initial weight} = 100 * TFI / IW,$

where TFI is total feed intake (g fish⁻¹ on as fed basis) and IW = initial body weight (g) of fish. One way ANOVA was used for testing possible differences in final individual fish weights, protein productive value (PPV) and daily feed intake (DFI), and the least significant differences (LSD) test was used for *post-hoc* comparisons.

3 RESULTS AND DISCUSSION

3.1 First feeding to post larvae (I)

3.1.1 Fractionation

The determination of fractionation through single diet feeding showed that the catfish reached asymptotic values before day 30 for FM and $\delta^{13}\text{C}$ of BNM and CM. However the $\delta^{15}\text{N}$ of the plant diets did not reach asymptotic values as at day 30 to enable for fractionation ($\Delta^{15}\text{N}$) of the catfish to be estimated. Based on the catfish stable isotope fractionation of nitrogen in BNM and CM (Fig. 4), it was evident that catfish $\Delta^{15}\text{N}$ of the BNM (c. 7 ‰) and CM (c.8 ‰) were high. To this effect fractionation was calculated as before stated and the estimated fractionation factors were $\Delta^{13}\text{C} \pm \text{S.D.} = -0.18 \pm 0.98 \text{ ‰}$, $\Delta^{15}\text{N} \pm \text{S.D.} = 3.3 \pm 0.61 \text{ ‰}$. These values are very close to typical values reported in the literature (Peterson & Fry 1987, Post 2002, Fry 2006). High fractionation of plant diets (>7 ‰) had been reported and also used in fractionation estimation, for soy protein ingredient in a two protein sources diets fed Pacific white shrimp (*Litopenaeus vannamei*) (Gamboa-Delgado & Le Vay 2009). Similarly in another two protein sources feeding experiment Martínez-Rochas et al. (2012) reported $\Delta^{15}\text{N}$ of 7.4 ‰ for pea meal (*Pisum sativum*) fed to Pacific white shrimp after 30 days. Usage of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values that are not fully asymptotic in estimating fractionation gives defective assimilation results. More so, fractionation results are sensitive in analysing stable isotope data from multi-protein ingredients sources diet using SIAR (Parnell et al. 2010). This prompted the use of alternative means of $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ calculation from the averages of mixed diets in this research.

The isotopic shift of consumer from diets has generally been estimated to be 0–1 ‰ for ^{13}C and 3.4 ‰ for ^{15}N (Owens 1987, Fry 2006). However in aquaculture using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for analyses of nutrient dynamics, the fractionation value of fish has to be known per ingredient or feed type. The extent of fractionation depicts the quality and quantity of food a consumer would have assimilated (DeNiro & Epstein 1981, Hobson 1999).

3.1.2 Growth and survival

The catfish larvae readily accepted the dry diets and those fed with F1 had the highest SGR, 8.52 % day⁻¹. The SGR of larvae fed with F1 was greater than those fed with F2 to F4 and all differed significantly ($P < 0.05$). African catfish grew well on our diet when compared to catfish of similar size fed with formulated dry diets SGR 10.6 % day⁻¹ (Chepkirui-Boit et al. 2011). However Chepkirui-Boit et al. (2011) noted better growth of catfish larvae fed with live diets like artemia or shrimp nauplii. It is noteworthy that the feed that was used in this experiment contained no vitamin premix or fish oil. The growth rates of the larvae enabled incorporation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ dietary isotopic signatures by tissue accumulation instead of tissue metabolic replacement (turnover). High somatic growth rate has been reported to be responsible for incorporation of $\delta^{15}\text{N}$ in brown shrimp (*Penaeus aztecus*) (Fry & Arnold 1982), isotopic signatures in broad whitefish (*Coregonus nasus*) (Hesslein et al. 1993), in posthatch age-0 smallmouth bass (*Micropterus dolomieu*) (Van der Zanden et al. 1998), in red drum (*Sciaenops ocellatus*) (Herzka & Holt 2000) and in larval Senegalese sole (*Solea senegalensis*) (Gamboa-Delgado et al. 2008). Conversely, in very slow growing catfish (*Pterygoplichthys disjunctivitus*) (a siluriformis catfish related to African catfish), fed with wood detritus diet, SGR was negligible (0.0017 % day⁻¹) and incorporation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ occurred by tissue turnover (German & Miles 2010).

The significant difference between the SGR of larvae fed F1 and those fed F2 ($P < 0.05$) was not surprising since the C: N-ratios of F1 was lower than in F2 (Fig. 5, see Table 3 (I), F1 C:N=7.01, F2 C:N=8.68). The crude protein content of F1 and F2 were also higher than in F3 and F4. The catfish fed with F4 had the lowest SGR (Table 6, Fig. 5). However it is important to note that the experiment was primarily designed to ascertain assimilation of ingredients and their biomass contribution without balancing the proteins or amino acid content. The supplementation of FM with BNM or CM coincides with reduction in dietary C:N-ratio of the feed. The high C:N-ratio of diets with higher BNM and CM inclusion could therefore be attributed to the much lower protein content and different amino acid compositions of BNM and CM as alternatives to FM in the larval diets. High diet C:N-ratio has been noted to be inversely proportional to diets nutritional value (Burns & Walker 2000, Piola et al. 2006). The catfish SGR in this experiment was negatively related to the C:N-ratio of the feeds F1-F4 (Fig. 5). The larvae fed with F2 (40 %BNM/40 %FM/20 %CM) had higher final weight and weight gain than F4 (60 %CM/20 %FM/20 %BNM). Final weight of larvae fed F1 (60 % FM) was higher than those F3 (60 %BNM) and F4 (60 %CM), suggesting fractionation effects of the high amount (60 %) of BNM and CM ingredients in the feeds. The final weight of those fed with F2 was similar to F3. This is not surprising since the amino acid profile of the diets F1 and F2 could have supported higher growth rate than in other diets with lower FM inclusion levels. The growth of the fish fed F2 also suggests that BNM cannot be included in the diets of larval African catfish at high level of 40

% without negative growth effect. Inclusion of 40 % BNM in larval catfish diet may be possible with amino acid supplementation. The low survival rate of the catfish in all diets could be due to use of dry diets in raising the catfish larvae. High mortality rate has been associated with using dry diet in raising larval African catfish (Segner et al. 1993, Hecht 1996). Moreover our diets did not contain vitamins and mineral which further lowered the nutritional value of the diets. There were minimal instances of agonistic behaviour but this was reduced because of feeding rate. Agonistic behaviour was in the form of larvae biting others at rest and inflicting injuries. In some cases the barbells are bitten off, thereby reducing food competitiveness of the fish (Hecht & Appelbaum 1998, Mukai et al. 2008). The injuries inflicted on the body exposes the fish to infections and barbells that are bitten off reduce feeding rate and they subsequently die (Hecht & Appelbaum 1998, Mukai et al. 2008, Mukai & Lim 2011). This potential problem was however anticipated, for which reason the larvae were fed four times a day. Frequent feeding has been noted to increase survival by reducing agonistic and cannibalistic behaviour in African catfish (Hecht & Pienaar 1993, Al-Hafedh & Ali 2004). However, the lower survival of catfish fed with F1 compared to F3 and F4 could be due to agonistic behaviours of the catfish as they grew up. Density related agonistic behaviour coupled with stress, could as well be responsible. In African catfish agonistic behaviors occur in form of individual fish biting each other at rest and in some cases cutting off barbells of others. The combined effects of exposed wounds and disability to compete for food due to cut barbells are deleterious. Agonistic behaviour and mortality had been reported in larval African catfish raised in tanks (Kaiser et al. 1995). This may not be very problematic anyway in a well-managed catfish culture where grading would be used to separate the fish of different sizes. Dead larvae as result of either nutritional or agonistic causes were counted. The number of dead fish was deducted from the living in calculating the growth and nutritional parameters. Dead fish were always removed before feeding 4 times a day. Constant provision of food was able to reduce individual attacks.

The larvae that were fed with sole ingredient FM grew better than those fed with either BNM or CM. This is expected anyway since FM is excellent feed ingredient for fish compared to the plant proteins.

TABLE 6 Initial weight, final weight, specific growth rate (SGR), average weight gain(AWG), and survival of larval African catfish fed for 30 days with diets F1 to F4 varying in fishmeal (FM), bambaranut meal (BNM) and corn meal (CM) (Table 1). The larvae fed with single diets (FM, BNM and CM) were weighed individually and analyzed separately from the fish fed with mixed diets. Values are means \pm SD (n=5) (I). Values within a column not followed by similar superscript are significantly different ($P < 0.05$).

Feed	Initial wt (g)	Final wt (g)	SGR (%)	AWG (g)	Survival (%)
F1	0.013 \pm 0.0 ^{ns}	0.168 \pm 0.02 ^a	8.52 \pm 0.46 ^a	0.155 \pm 0.02 ^a	36.66 \pm 20.05 ^b
F2	0.012 \pm 0.0 ^{ns}	0.114 \pm 0.01 ^{ab}	7.44 \pm 0.39 ^b	0.102 \pm 0.01 ^b	49.16 \pm 12.58 ^{ab}
F3	0.012 \pm 0.0 ^{ns}	0.086 \pm 0.01 ^b	6.59 \pm 0.57 ^c	0.075 \pm 0.01 ^c	60.00 \pm 8.66 ^a
F4	0.014 \pm 0.0 ^{ns}	0.071 \pm 0.02 ^c	5.34 \pm 0.36 ^d	0.057 \pm 0.02 ^d	58.33 \pm 18.05 ^a
FM	0.026 \pm 0.0 ^{ns}	0.085 \pm 0.04 ^A	3.92 \pm 0.02 ^A	0.059 \pm 0.01 ^A	-
BNM	0.013 \pm 0.0 ^{ns}	0.024 \pm 0.02 ^B	2.01 \pm 0.01 ^B	0.011 \pm 0.03 ^B	-
CM	0.012 \pm 0.0 ^{ns}	0.016 \pm 0.01 ^C	0.82 \pm 0.03 ^C	0.003 \pm 0.01 ^C	-

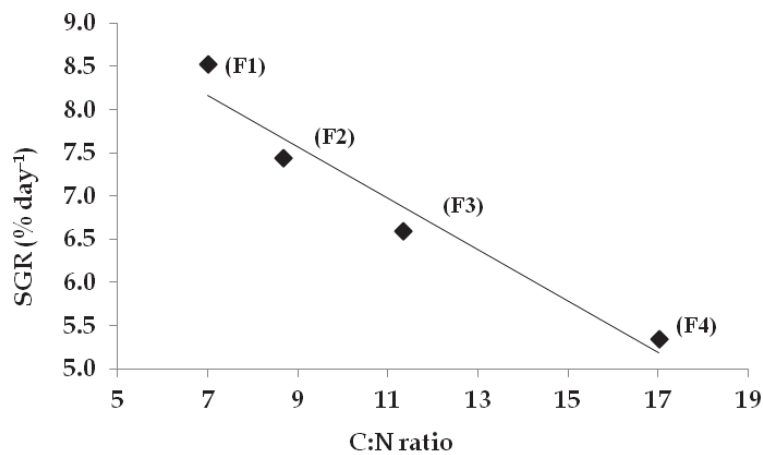


FIGURE 5 Relationship of specific growth rate (SGR) of African catfish larvae and the feed carbon:nitrogen (C:N) ratios after 30 days feeding with diets varying in content of fishmeal, bambaranut meal and corn meal (Table1). The catfish SGR values represent averages of three replicated tanks per feed type (I).

3.1.3 Stable isotope analyses

The three feed ingredient sources (BNM, CM and FM) had divergent isotope signatures (Figs. 1 & 6). The divergent stable isotopes signature values enabled clear construction of the model without value overlap. CM had typical $\delta^{13}\text{C}$ values for a C4 plant source (from -10 ‰ to -17 ‰) while BNM values reflected a C3 plant source (from -22 ‰ to -35 ‰) (Tiunov 2007). Bambaranut has been always regarded as tropical plant of African origin (Dakora & Muofhe 1995,

Okonkwo & Opara 2010). However the isotope signature result of bambaranut shows it may not originally be of African origin. Bambaranut may have been introduced into Africa by early explorers. On the other hand, if bambaranut is of tropical African origin, then our result suggests that it may be an outlier in the continuum of C3–C4 plants classification. However there was divergence in the isotope signatures of CM and BNM thereby enabling the model biplot. In previous experiments different isotopic shift (fractionation) was identified in Nile tilapia by use of C3 feed ingredient (wheat) and C4 (corn) (Focken 2004). Similarly by culturing soil nematode (*Panagrellus redivivus*) on a C3 plant (wheat) and C4 (corn), larval fish diets with divergent isotopic signatures were obtained (Schlechtriem et al. 2004).

In this thesis larval catfish isotope signatures resembled that of their respective diet (from where the isotopic signatures were incorporated) (Fig. 6). (Note that isotope signatures in Fig. 6 have been corrected for fractionation). Consequently, the isotope signatures of the larvae fed with F1 orientated towards FM. The larvae fed with F3 orientated more towards BNM and those on F4, more towards CM (Fig. 6). The orientation of the larval isotope signatures within the model shows fast incorporation of dietary isotopes signatures within research period. This holds true since first feeding larval catfish were used in the experiment and their first and only external food were they experimental diets. The observed nitrogen contribution of the ingredients to catfish crude protein showed that FM contributed most in F1 while BNM and CM contributed more in F3 and F4, respectively (Table 7).

Table 7. Nitrogen contribution from bambaranut meal (BNM), corn meal (CM) and fishmeal (FM) to the somatic crude protein of larval African catfish fed diet mixes F1-F4.(See Table 1 for diet composition)(I).

	F1 (%)	F2 (%)	F3 (%)	F4 (%)
BNM	8.71	13.14	22.94	10.92
CM	3.46	4.10	4.08	7.72
FM	42.32	38.56	27.60	35.39
Total	54.49	55.79	54.63	54.03

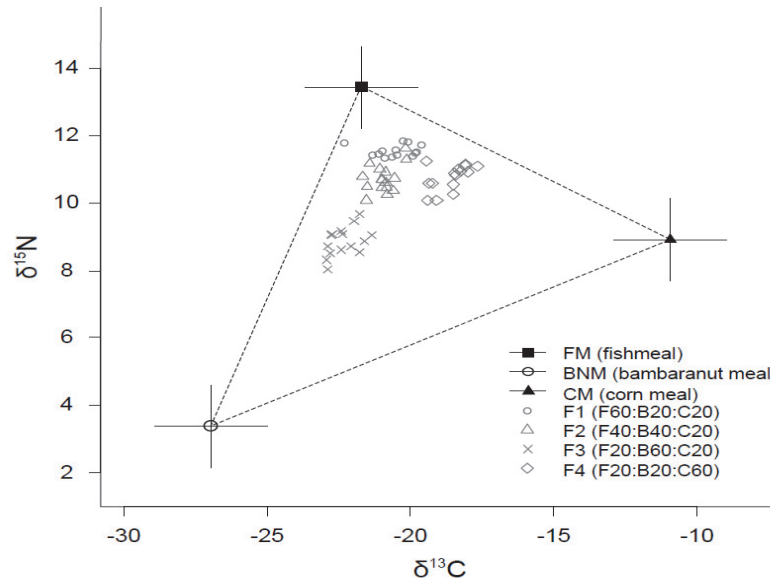


FIGURE 6 Stable isotope signature bi-plot of fishmeal (FM), bambaranut meal (BNM) and corn meal (CM) and for larval African catfish at the end of a 30-day feeding experiment with four experimental diets feeds (F1 to F4) varying in amount of FM, BNM and CM inclusion levels (see Table 1 for feed composition). F1 to F4 represent feed 1 to feed 4. Note that isotope values for feed ingredients have been corrected for trophic fractionation ($\Delta^{13}\text{C} \pm \text{S.D.} = -0.18 \pm 0.98$, $\Delta^{15}\text{N} \pm \text{S.D.} = 3.3 \pm 0.61$), error bars of single diets represents S.D (n=39, data points represents each of five individuals analysed (see 3.1.1) (I).

3.1.4 Assimilation of nutrients and biomass contributions

Based on the outputs of the SIAR model, biomass of catfish fed with F1 constituted of 43.4 % nutrient assimilated from FM and 30.2 % assimilated from BNM. Irrespective of the fact that CM was included at the same quantity as BNM its contribution to biomass was lower at 26.2 % (Fig. 7 A). Assimilation of nutrients from FM and BNM in F2 (FM 40 % and BNM 40 %) was close for both FM (34.2 %) and BNM (39.1 %) but 26.6 % from CM (Fig. 7 B). Although the catfish assimilated as much nutrient from BNM, the nitrogen contribution from FM to the fish crude protein was higher than from BNM (Table 7). However the nitrogen contribution to the catfish crude protein from BNM in F2, was higher than from F1. This could be due to increased availability of some essential amino acids as BNM inclusion level increased to 40 % from 20 % of F1. At 60 % BNM inclusion (F3) the catfish assimilated more nutrients from BNM (57.4 %), than both FM (20.4 %) and CM (22.2 %) (Fig. 7 C). Similarly the observed nitrogen contribution of BNM from F3 (22.94 %) to the crude protein of the catfish was close to that from FM (27.60 %). The increased assimilation of

nutrient from BNM and increased contribution of nitrogen could be because of increased level of amino acids like lysine. Most nitrogen (N) present in animal is in form of amino acids (Schoeller 1999). Assimilation of dietary essential amino acids in fish has been noted to be with little or no fractionation (McMahon et al. 2010). Although the catfish assimilated more nutrient from BNM in F3, the growth rate was lower than in the catfish fed F1 (60 % FM) or F2 (40 % FM and BNM). This suggests effects of non protein components which may have reduced growth alongside. The catfish assimilation of nutrients from F4 was highest for CM (44 %), but similar for BNM (27.5 %) and FM (28.6 %) (Fig. 7D). Although the catfish assimilated more nutrients from CM in F4, nitrogen contribution was lowest from CM (7.72 %) but highest from FM (35.39 %). The poor growth of the catfish fed with F4 could be due to the low levels of essential amino acid in CM. In as much as FM contributed more nitrogen than BNM and CM to the catfish fed with F4 (Table 7), the inclusion level of FM and BNM were low. The low inclusion level of FM and BNM coupled with assimilated non protein nutrients like carbohydrates from CM in F4 may have reduced growth rate. Inclusion of 30 % BNM to maize meal has been noted to improve its nutritional quality (Mbata et al. 2009). However poor growth of the catfish could also be due to the high dietary C:N-ratio and low oil content of the diets. The catfish were fed with diets that had no additional oil inclusion. Ingredients used in diet formulation (FM, BNM and CM) were low in oil content. With low lipid diets and larval active stage and high growth rate of larval fish, lipids may have been all used up and not stored. This could lead to utilization of dietary protein for energy production and hence poor growth rate of the catfish.

There was positive correlation between the observed and expected nitrogen contribution to the fish biomass (Fig. 8). The mean absolute error (MAE) of difference (between observed and expected values) was low (3.0) indicating that for every gram (g). of the observed nitrogen contribution there was 3 g difference with the expected value. The contribution from FM was highest and this is not surprising because FM is excellent ingredient in fish feed. However the contribution from BNM was high enough suggesting that BNM is plausible alternative to FM in diets of African catfish.

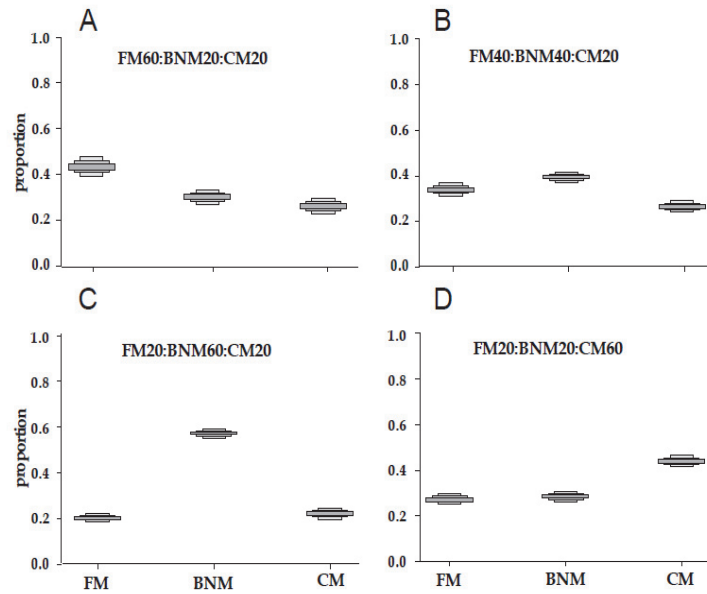


FIGURE 7 Results of SIAR isotope mixing model for larval African catfish proportional nutrient assimilation from fishmeal (FM), bambaranut meal (BNM) and corn meal (CM) in four different feed mixtures as tabulated in Table 1. The three bars represent SIAR results at 95%, 75% and 25% credibility intervals (I).

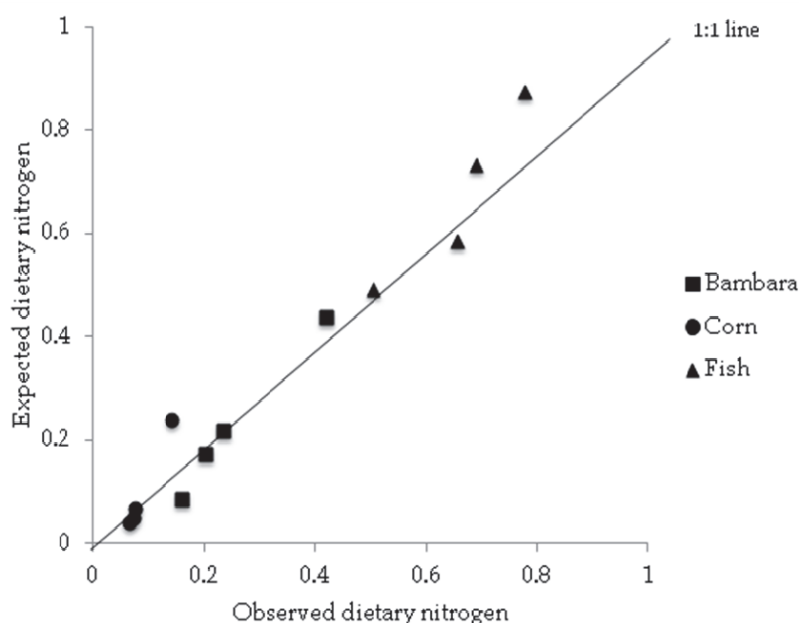


FIGURE 8 Expected (based on feed composition) and observed (measured values) nitrogen contribution from the three major feed ingredients, fishmeal, bambaranut meal and corn meal, to fish biomass ($y = 1.065x - 0.022$, $R^2 = 0.97$, $MAE = 3.001$). Square= contributions from BNM, circles= contribution from CM, and triangle from FM. Data points represent averages from three replicates ($n=5$) (I).

3.2 Post larvae to fingerling (II)

3.2.1 Growth, survival and nutritional parameters

The catfish grew fast on the experimental diets attaining average tankwise SGR between 13.1 % and 14.9 % day^{-1} . There were no significant differences in the SGR of catfish fed with diets F1 (25 % SBM, 5 %BNM) to diet F3 (15 % SBM, 15 %BNM) ($P > 0.05$). The catfish fed with F4 (10 % SBM, 20 % BNM) and F5 (5 % SBM, 25 % BNM) were similar in SGR but different from those of F1 to F3 ($P < 0.05$). The catfish SGR was reduced with addition of up to 25 % BNM (F5) or 20 % (F4) in the diet (Table 8). This could be due to higher amount of carbohydrate in BNM than SBM. Bambaranut meal is known to contain about 50 % carbohydrates (Amadi et al. 1999, Yusuf et al. 2008). African catfish utilizes carbohydrates well (Ali & Jauncey 2004). However, at the developmental stage we experimented they may not have utilized carbohydrates well, leading to reduced growth as BNM increased. This suggests that BNM cannot be included in diets of larval African catfish beyond.

Food conversion ratio (FCR) was below 0.9 for all diets. Catfish FCR was similar for those fed F1 and F2 ($p>0.05$) and F3 and F4 ($p>0.05$), but all better than F4 (Table 8). Although there was little but significantly elevated FCR with BNM addition the growth and FCR of the catfish were still good, suggesting effects of amino acids in the diets. The amino acid of the diets (II), contained high amount of growth promoters like lysine and methionine. The diets contained high amount of fishmeal which could have also enhanced supply of needed essential amino acids. Amino acid content of the diets was also up to the recommended level (II). The protein efficiency ratio and the protein retention of the catfish were positively correlated with increasing inclusion of SBM in the diet (Fig. 9).

Survival of the catfish did not seem to follow increase of either SBM or BNM (Fig. 10). However there was also a sharp drop in catfish survival at F3 and the cause is not very clear, but could have been by chance as well. The catfish usually attack one another (agonistic behavior) in resting positions inflicting wounds and cutting off barbells of others (Hecht & Appelbaum 1998, Mukai et al. 2008). Barbells are important in food location and competition. The incidence of injury and cut barbells reduces the catfish feeding ability, exposes them to diseases and eventually death. Kaiser et al. (1995 a, b) noted that catfish needs stocking density of 0.6 fish cm^{-2} which reduces agonistic behavior. However, depending on their age, larval African catfish do not swim on the bottom always (especially at early stages) (Mukai & Lim 2011). Mortality could therefore be due to combination of factors like aquarium bottom density effects, use of dry diets and tendency to over feed. Dry diets have been noted to cause high mortality in African catfish (Chepkirui-Boit et al. 2011). However mortality reduced number of fish and there was higher final catfish weight of the F3 treatment. Besides the two replications design could have also contributed to mortality record, not being enough to determine over all causes. The mortality due to agonistic behavior can easily be controlled in a culture system by means of grading.

TABLE 8 Average (\pm SD) (n=4) initial weight (g), final weight (g) specific growth rate (SGR %day⁻¹), feed conversion ratio, of African catfish post larvae fed diets that varied in amount of soybean meal (SBM) and bambaranut meal (BNM) as substitutes of fishmeal (FM) (II). Values within a column not followed by similar letters are statistically significantly different $p<0.05$.

Feeds	Initial wt	Final wt	SGR	FCR
F1	0.12 \pm 0.01 ^{ns}	2.90 \pm 0.03 ^c	14.93 \pm 0.31 ^a	0.66 \pm 0.10 ^a
F2	0.14 \pm 0.03 ^{ns}	3.33 \pm 0.06 ^b	14.31 \pm 0.81 ^a	0.68 \pm 0.01 ^a
F3	0.16 \pm 0.00 ^{ns}	4.30 \pm 0.05 ^a	14.89 \pm 0.00 ^a	0.74 \pm 0.00 ^b
F4	0.17 \pm 0.00 ^{ns}	3.06 \pm 0.36 ^c	13.13 \pm 0.35 ^b	0.77 \pm 0.03 ^b
F5	0.15 \pm 0.00 ^{ns}	3.00 \pm 0.17 ^c	13.59 \pm 0.20 ^b	0.84 \pm 0.06 ^c

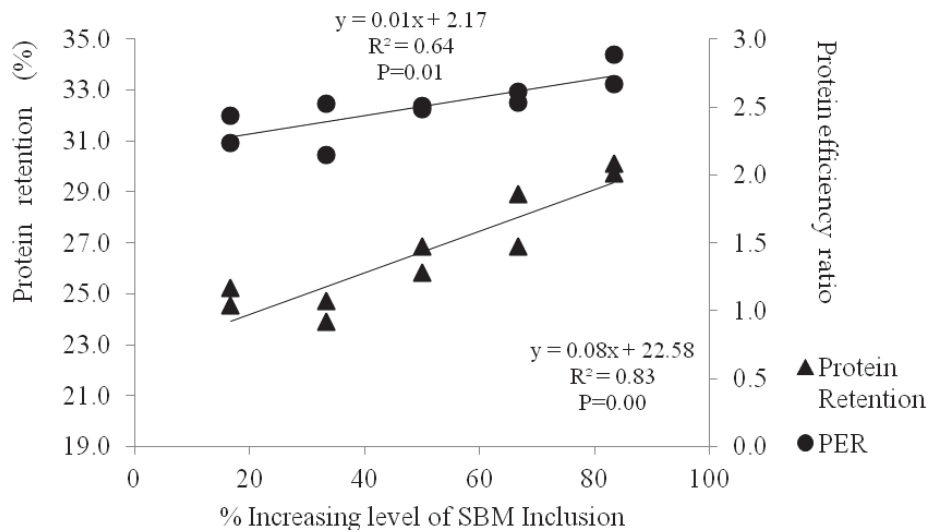


FIGURE 9 Relationship of protein retention, protein efficiency ratio (PER) of African catfish fed diets varying in content of soybean meal (SBM) with bambaranut meal (BNM). The % inclusion of soybean meal per feed type are F1= 83.33 %, F2 = 66.67 %, F3 = 50 %, F4 = 33.33 % and F5 = 16.67 %. The reverse of these values are % inclusion of bambaranut meal. Data points represent averages from each of the two tanks (II).

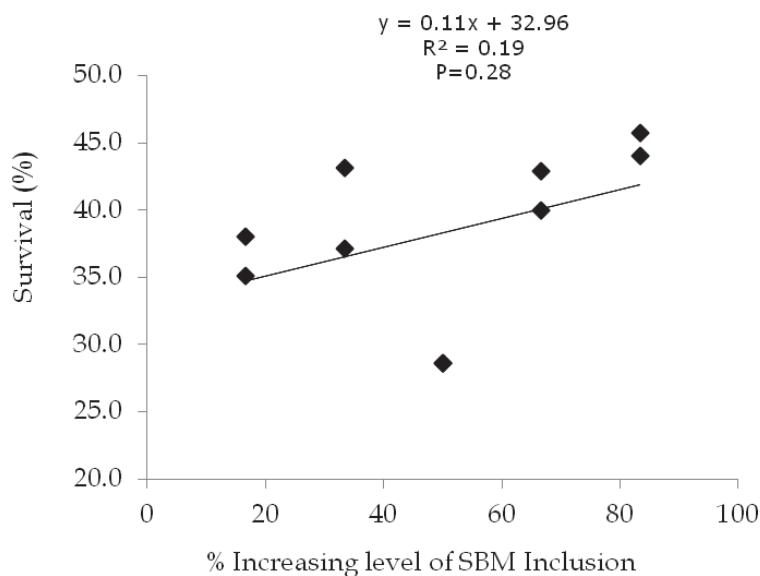


FIGURE 10 Relationship of survival of African catfish with increasing soybean meal (SBM) inclusion after feeding experiments with diets varying SBM with bambaranut meal (BNM). Values on the x-axis shows SBM share of the SBM:BNM mixture (II).

TABLE 9 Average \pm SD (n=4) growth and nutritional parameters of Africancatfish fed diets F1 - F4 for 22 days varying in sesame seed meal and bambaranut meal content as in Table 3. IW = initial weight (g), FW = final weight (g), AWG = average weight gain (g), SGR = specific growth rate (% day⁻¹), FCR = feed conversion ratio, PER = protein efficiency ratio, PR = protein retention efficiency, HSI = hepatosomatic index, FSI = peritoneal fat somatic index, TFI = total feed intake (g fish⁻¹) and Cost of feed as USD per kg. ECR= economic conversion ratio, C.Prot = Cost per unit per kg protein of catfish produced (\$). Means within a row not followed by the same superscript are significantly different (P<0.05), ns = no significant difference (III).

Parameters	F1	F2	F3	F4
IW	11.69 \pm 1.00 ^{ns}	11.31 \pm 0.14 ^{ns}	11.45 \pm 0.04 ^{ns}	11.71 \pm 0.33 ^{ns}
FW	61.94 \pm 3.62 ^b	72.11 \pm 7.62 ^a	70.66 \pm 3.94 ^a	72.51 \pm 8.73 ^a
AWG	50.28 \pm 3.00 ^a	60.80 \pm 7.65 ^b	59.21 \pm 3.99 ^b	60.79 \pm 8.59 ^b
SGR	7.60 \pm 0.27 ^b	8.34 \pm 0.51 ^a	8.57 \pm 0.27 ^a	8.67 \pm 0.54 ^a
FCR	0.80 \pm 0.04 ^b	0.73 \pm 0.05 ^a	0.71 \pm 0.04 ^a	0.72 \pm 0.03 ^a
PER	2.76 \pm 0.15 ^b	2.99 \pm 0.20 ^{ab}	3.07 \pm 0.18 ^a	3.10 \pm 0.11 ^a
PR	56.47 \pm 0.31 ^a	54.86 \pm 5.50 ^a	57.23 \pm 1.05 ^a	55.44 \pm 5.70 ^a
HSI	1.36 \pm 0.21 ^a	1.17 \pm 0.03 ^b	1.14 \pm 0.11 ^{bc}	0.96 \pm 0.18 ^c
FSI	1.14 \pm 0.16 ^{ns}	1.24 \pm 0.27 ^{ns}	1.36 \pm 0.44 ^{ns}	1.34 \pm 0.32 ^{ns}
TFI	38.89 \pm 0.83 ^a	43.59 \pm 2.91 ^a	41.30 \pm 1.58 ^a	43.28 \pm 2.7 ^a
Cost	1.50 \pm 0.08 ^b	1.55 \pm 0.10 ^b	1.59 \pm 0.08 ^{ab}	1.64 \pm 0.04 ^a
ECR	1.16 \pm 0.06 ^{ns}	1.13 \pm 0.08 ^{ns}	1.13 \pm 0.07 ^{ns}	1.17 \pm 0.58 ^{ns}
C.Prot	0.023 \pm 0.01 ^{ns}	0.024 \pm 0.04 ^{ns}	0.024 \pm 0.10 ^{ns}	0.024 \pm 0.03 ^{ns}

3.3 Fingerlings to juvenile (III)

3.3.1 Growth and nutritional parameters

The catfish readily accepted the experimental diets and grew very fast with SGR that varied from 7.60 \pm 0.27 % day⁻¹ (F1, BNM 35 %: SSM 0 %) to 8.67 \pm 0.54 % day⁻¹ (F4, BNM 0 %: SSM 35 %). There were no significant differences (P>0.05) in the SGR of catfish fed F2 (BNM 23.3 %: SSM 11.7 %), F3 (BNM 11.7 %: SSM 23.3 %) and F4 but those fed F1 grew significantly slower (Table 9). SGR was similar for all diets containing sesame seed meal (SSM). Although SSM contains less lysine than bambaranut meal it has more methionine. Low content of lysine in SSM diets may have been compensated by the high fishmeal inclusion which enhanced growth. High dietary lysine has been noted to improve feed intake in African catfish (Ozório et al. 2002, Davies & Ezenwa 2010). The catfish fed with diets having SSM alone or included with BNM (F2–F4) grew much faster than those on FM–BNM (F1) diet, the SGR of those on F1 (7.60 \pm 0.27 % day⁻¹) was better than that of catfish of similar age reported in Davies & Ezenwa (2010). This indicates that catfish also grow relatively well on FM–BNM diet. High FM level in the diets have provided essential amino acids at levels likely meeting catfish requirements. The FCR of the catfish was \leq 0.8 for

all treatments. The fish fed with F2 (BNM 23.3 %/ SSM 11.7 %), F3 (BNM 11.7 %/ SSM 23.3 %) and F4 (BNM 0 %/ SSM 35 %) with average FCR 0.72, had significantly lower ($P < 0.05$) FCR than those fed F1 (BNM 35 %/ SSM 0 %) FCR, 0.80 ± 0.04 . The dietary content of essential amino acids (EAA), like lysine and methionine, may have promoted growth and feed utilization of the catfish. However, the catfish consumed more of F1 than F3 and F4 (Table 9). The cost of the feed was significantly higher for F4 and F3 compared to F2 and F1. Feed costs increased with SSM inclusion.

3.3.2 Stable isotope analyses, assimilation and biomass contributions

The isotope signatures of catfish fed with three protein source diets orientated within the perimeters of the SIAR model plot (Fig. 11). The signature of the larvae fed with F3 (17.7 % BNM, 23.3 % SSM and 53 % FM) orientated more towards FM since the catfish assimilated more nutrients from FM than SSM (Fig. 11). Similarly those fed with F2 (23.3 % BNM, 17.7 % SSM and 53 % FM) orientated closer to FM and SSM than BNM, since the catfish assimilated more SSM nutrient than BNM (Table 10) (see Table 3 for feed composition). The catfish fed with two protein sources diet (F1, 35 % BNM and 53 % FM), assimilated nearly similar amount of nutrients from both FM (51.34 %) and BNM (48.66 %) (Table 10). However the nitrogen contribution to the crude protein from the FM (49.98 %) was much higher than from BNM (14.69 %). Similarly the catfish fed with reciprocal SSM diet F4 (35 % SSM and 53 % FM) assimilated 59.52 % FM nutrients and 40.48 % of SSM. The nitrogen contribution from FM (56.35 %) was higher than from SSM (12.71 %). Catfish higher nutrient assimilation from BNM than SSM could be due to the amino acid like lysine and isoleucine that are more in BNM than SSM. The diet combination of SSM and FM enhanced more assimilation from FM than BNM. The reason is not clear but maybe due to some non protein dietary components like lipids in SSM and carbohydrates in BNM. The catfish nutrient assimilations from three protein sources diets were different from the two sources. However, the catfish assimilated more nutrients from SSM (41.46 %) than BNM (19.96 %) in F2 (Fig. 12A). Nutrient assimilation from FM in F2 was (44.51 %). The nitrogen contribution from FM to crude protein was 44.81 % while it was 13.84 % for SSM and 6.23 % for BNM. The higher nutrient nitrogen contribution from SSM than BNM could be because more SSM nutrient than BNM was assimilated from F2. The catfish fed with F3 assimilated more nutrients from FM (50.43 %) than BNM (14.37 %) and SSM (42.65 %) (Fig. 12B). There was also similar amount of nitrogen contributed from SSM as in F2 but lower nitrogen contribution from BNM. This could be because BNM inclusion level in F3 was lowest. Reason could as well be due to high lipid content of SSM than BNM and lesser amount of carbohydrates in SSM than BNM in the mixture. These stated reasons for higher assimilations of nutrients, need further research (example nutritional biochemistry responsible) for clarification, because there could be some nutrient interactions of the three ingredient components of F2 and F3.

The quantities and sources of nutrients assimilated by the catfish could be reasons for the growth differences of the catfish fed these diets. There was increase in catfish $\delta^{15}\text{N}$ from F1-F4 and C:N-ratio from F1 to F3 and it was lowest in F4 (Table 11). The FCR of the catfish was similar for those fed feed F2, F3 and F4. This goes to show also that the higher growth rate of catfish fed diets F2 to F4 fed catfish was not entirely due to the SSM alone, but also as result of high FM assimilation from the diets. The result also shows that lower SGR of F1 compared to F2-F4 could be attributed to nutrient assimilated and maybe interaction effect in the FM-BNM and FM-SSM diet complexes.

TABLE 10 Crude protein (C.protein)%, % nitrogen contribution (N contribution) from bambaranut meal (BNM), fishmeal (FM) and sesame seed meal (SSM) and the % assimilated nutrients per ingredient in feeds of African catfish fingerlings fed diets varying FM, BNM and SSM (see Table 3 for feed composition) (III).

Nutrient assimilations from ingredients	C. protein			N contribution			
	% FM	% SSM	% BNM	%N*6.25	%BNM	%FM	%SSM
F1	51.34		48.66	64.68	14.69	49.98	
F2	44.51	41.46	19.96	64.89	6.23	44.81	13.84
F3	50.43	42.65	14.37	67.21	4.34	49.10	13.77
F4	59.52	40.48		69.07		56.35	12.71

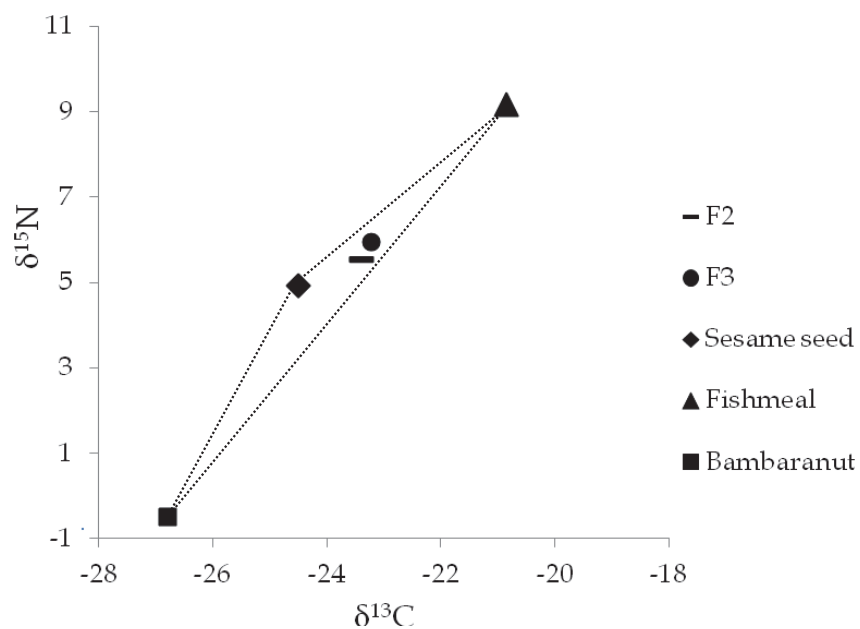


FIGURE 11 Stable isotope signature bi-plot of feed ingredients (fishmeal, bambaranut meal and sesame seed meal), and fingerling Africa catfish after 28-days feeding experiment with three protein sources diets (F2 and F3) varying in FM, BNM and SSM inclusion levels. See Table 3 for details of feed composition. Note that isotope values for feed ingredient have been corrected for trophic fractionation as in Fig. 6 (III).

TABLE 11 The $\delta^{13}\text{C}$ (‰), $\delta^{15}\text{N}$ (‰), %C, %N and C:N ratio of African catfish larvae fed with diets varying sesame seed meal, bambaranut meal and fishmeal and isotopic signatures of the ingredients. See Table 3 for ingredient compositions per diet (III). Values represent means \pm SD. Values in same column not followed by same superscript are significantly different ($p < 0.05$).

Feed	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	%C	%N	C:N
F1	-24.17 ± 0.45^a	10.35 ± 0.50^a	53.33 ± 2.95^c	8.63 ± 0.89^b	6.17^c
F2	-23.95 ± 0.23^b	10.38 ± 0.15^a	54.81 ± 2.45^b	8.71 ± 0.49^b	6.29^c
F3	-23.87 ± 0.27^b	10.75 ± 0.05^b	55.85 ± 2.22^a	8.34 ± 0.72^b	6.70^b
F4	-23.67 ± 0.25^b	11.05 ± 0.10^c	55.65 ± 2.11^a	9.21 ± 0.72^a	6.00^a
SSM	-24.50 ± 0.12^B	4.94 ± 0.06^B	59.29 ± 0.05^A	3.96 ± 0.04^B	14.96^C
BNM	-26.79 ± 0.04^C	-0.49 ± 0.02^C	45.83 ± 0.09^B	3.70 ± 0.04^B	12.37^B
FM	-20.85 ± 0.01^A	9.17 ± 0.02^A	45.44 ± 0.01^B	11.95 ± 0.07^A	3.80^A

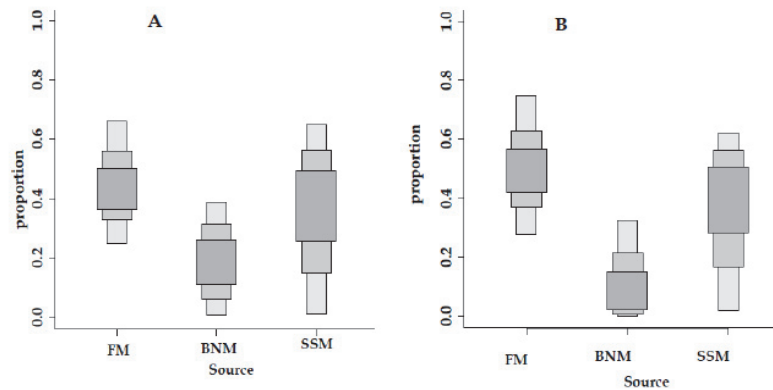


FIGURE 12 Assimilation of nutrients from three protein sources diets varying in fishmeal (FM), bambaranut meal (BNM) and sesame seed meal (SSM) contents. (A) Assimilation from feed 2, (B) assimilation from feed 3. The three bars represent SIAR results at 95 %, 75 % and 25% credibility intervals. (see table 3 for feed composition) (III).

3.4 Fingerlings to juvenile (IV)

3.4.1 Growth and nutritional parameters

The catfish SGR increased with increasing inclusion of FM. However substitution of 50 % FM with BNM (F5, 30 % BNM: 30 % FM) or SBM (F6, 30 % SBM: 30 % FM) produced the best SGR among the catfish fed with substitution

diets. The best SGR of catfish was by those fed 60 % FM diet (F3) (c. 5% day⁻¹) compared to those F5 (c. 3.7 % day⁻¹) or F6 (c. 3.5 % day⁻¹) (See IV). The result of FM substitution with 300 g kg⁻¹ SBM and BNM agrees with previous findings of Imorou Toko et al. (2008), who noted that SBM can replace FM up to 300 g kg⁻¹. However complete replacement of FM with plant proteins in this thesis (F4 BNM 30: SBM 30) reduced SGR to 2 % day⁻¹.

Food conversion ratio (FCR) of the catfish followed same trend as the SGR (IV). The catfish fed with F3 had lowest FCR and FCR increased with the increase of plant proteins. This could be due to the reducing amino acid levels of the diets as FM is substituted with both BNM and SBM. The catfish FCR was close for those fed F5 (1.1) and F6 (1.0). Similarly there was similar linear increase in condition factors (CF) of the catfish at all levels of BNM, SBM substitution of FM.

There was however linear (but small) increases in the catfish CF with increasing inclusion of FM in diets. This goes to show that although catfish grew very well at high (60 %) FM inclusion, substitution with 50 % BNM or SBM was economically comparable though with growth trade-offs.

The economic conversion ratio favours reduction of fishmeal therefore making it uneconomical to use F3 (ECR >1), even though it had the highest SGR. The ECR of F5 was 0.6 USD kg⁻¹ while F6 was 0.7 USD kg⁻¹, which indicates that SBM was costlier than BNM (IV). The feed conversion ratio of the catfish followed the trend of the growth. The amino acid profile of the diets could be responsible for the catfish growth and nutritional responses. The result of SGR of the catfish fed with F5 and F6 shows that there was no difference in substituting FM with either BNM or SBM in diets of African catfish at this stage of development.

3.4.2 Assimilation of nutrients and stable isotope analyses

The catfish grew rapidly incorporating dietary isotopic signature by fast growth as opposed to turnover (Hesslein et al. 1993) (IV). The catfish isotopes signatures resembled that of their diets and were within the perimeters of the model (Fig. 13). Assimilation of nutrients from the two protein source diets showed variable assimilation for FM, BNM and SBM in the two protein sources diets (F4, F5 and F6). There was similar assimilation of nutrients from both BNM (49.52 %) and SBM (50.48 %) by the catfish fed feed F4. The catfish assimilated more BNM from F5 (61.42 %) than FM 38.58 %. Similarly the catfish fed with F6 assimilated more nutrients from SBM 56.98 % than FM 43.02 % (IV). This suggests some effects of nutrient interactions in the diet mixes FM-BNM compared to FM-SBM. However it also shows that African catfish may not need more than 30 % FM addition in diets of post fingerlings.

In as much as bambaranut is known to be richer than most other legumes in its content of essential amino acids like lysine and methionine (Dakora & Muofhe 1995), in this thesis it was evident that high BNM inclusion levels resulted in other amino acids' deficiency compared to SBM. Despite lower protein content in BNM than in SBM, and consequent sub-optimum amino acid

levels, supplementation of FM with either SBM or BNM produced similar growth rates (Fig.14). This can be because as the SIAR result showed, little more FM nutrients were assimilated in FM-BNM diets than in FM-SBM diet in the three protein sources.

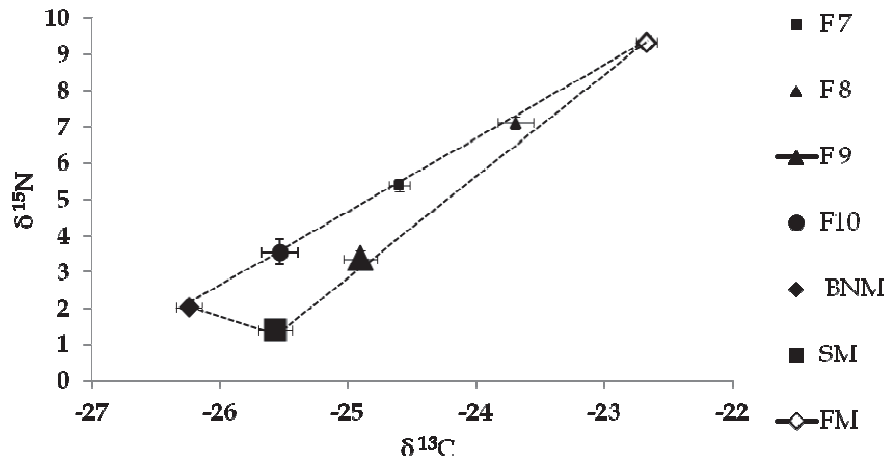


FIGURE 13 Isotope signature bi-plots for fishmeal (FM), bambaranut meal (BNM), and soybean meal (SBM), and for fingerling of African catfish at the end of feeding experiment, fed three protein source diets (F7 to F10) varying in FM, BNM and SBM inclusion levels. (See Table 4 for details of feed composition). Isotope values for feed ingredients have been corrected for trophic fractionation. The data points represent averages of five replicate samples from each treatment and error bars represent S.D. (IV).

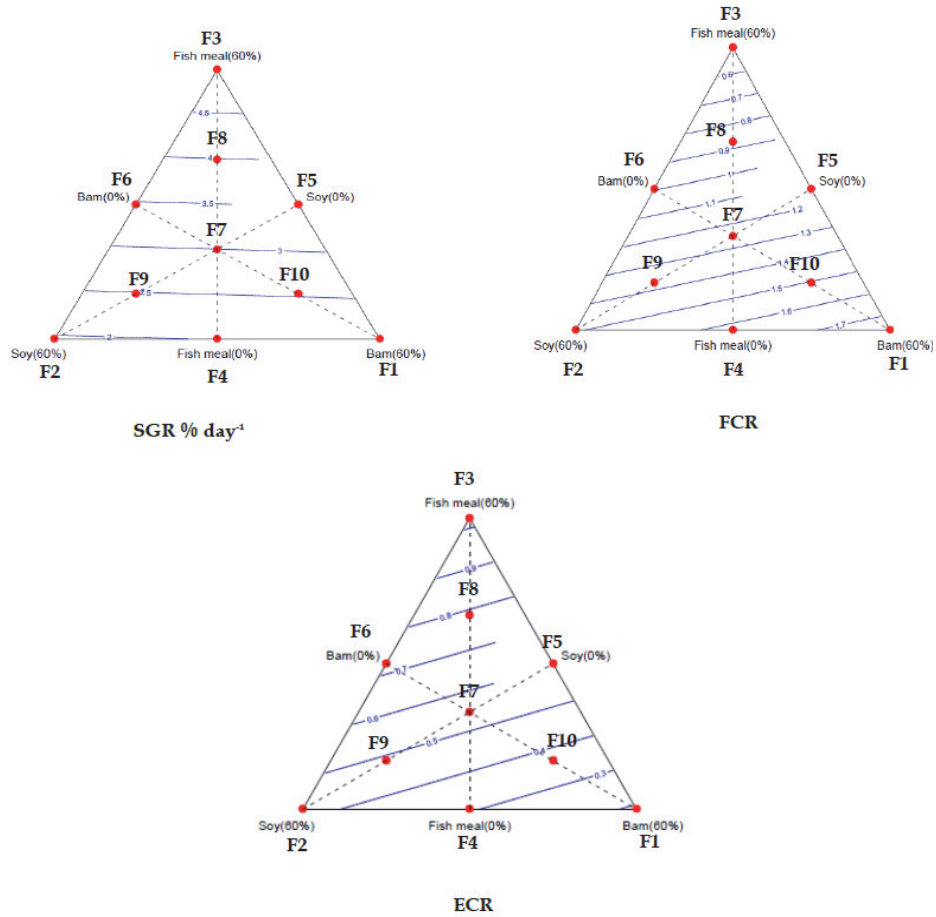


FIGURE 14. Specific growth rate (SGR), food conversion ratio (FCR) and economic conversion ratio (ECR) of African catfish fed with diets varying in soybean meal (soy, SBM), bambaranut meal (bam, BNM) and fishmeal (FM) contents. The feed points are denoted on the figure. The figures are read from 0 % to 60 % for each ingredient. That means, to read each feed item, the triangle is turned such that the 0 % is at the base while the 60 % is at the peak (IV).

4 CONCLUSIONS

The production of novel diets for African catfish was possible with plant protein (BNM, SBM, and SSM) substitutes of FM. Developmental stage of the catfish is important in considering and choosing ingredients and feed design. Although particular ingredients like BNM and CM may not be well utilized by the catfish at the larval or post larval stages, it could be well utilized in latter stages. The larval stage of the African catfish utilized less BNM than post larval and fingerling to juvenile stage. Consequently based on the growth and nutritional performances of catfish larvae fed with BNM diets in I and II BNM cannot be included beyond 25 % in larval diets without compromising growth. Assimilation of BNM in the larval stage (I) showed that it is a good supplement of FM at low inclusion level. The catfish however utilised SBM better at larval stage than BNM (II). Conversely, in fingerling stage BNM can completely replace SBM without negative growth effects. The growth and nutritional performances of catfish fed with SSM showed that it is a good substitute of FM and can completely replace BNM in catfish diet. Combination of SSM and FM enhanced assimilation of nutrients from FM by the catfish. SSM in combination with BNM is also a good supplement of FM producing very fast growth of the catfish. The poor catfish growth and nutritional performances with CM diets suggests that it cannot be used as alternative protein source for FM in catfish diets.

Results also showed that ingredients nutrient assimilation was proportional to fish biomass contribution. Analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the fish fed larval diets showed that the assimilated nutrients biomass contribution to catfish larvae from BNM was similar to FM. The biomass contribution of each tested feed ingredient increased with inclusion level. Similarly nitrogen contribution to crude protein of the fish increased with inclusion level. Assimilation and biomass contribution from ingredients in two and three protein sources diets were not the same. The developmental stage of catfish determines nitrogen requirement and contribution from ingredients. Larval stages of the catfish had more nutrient and nitrogen contribution from FM than fingerline and post fingerling stages. While FM was assimilated more at two protein sources diets, it was assimilated less at three protein sources diets in

(IV). Assimilation of FM in IV was lower than the plant proteins. The assimilation of FM was however higher than SSM at both two and three protein sources diets. This could be attributed to the high lipid content of the diets. The differences in assimilation of nutrients from the plant proteins suggest effects of ingredients' nutrients interactions. Effects of ingredients nutrients interactions are important in feed formulation. The tested plant proteins (BNM, SSM and SBM) were suitable fishmeal alternatives in formulating novel diets for African catfish. The lipid content of the diets had effects on the catfish lipid content. Consequently diets without fish oil caused low body lipid in the larvae. Stable isotope analyses can be invaluable guiding tool to ascertain assimilation and biomass contribution and effects of ingredients nutrients interactions in diet formulation.

This thesis contributes novel and cheap feed mixes that produced high SGR in African catfish. The issues of assimilation and nutrient interaction are as important as substitution of the ingredients and could be helpful in optimizing diet mixes.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Kasviproteiinien käyttö jättikonnamonnin *Clarias gariepinus* rehuissa

Jättikonnamonni *Clarias gariepinus* on afrikkalainen kalalaji, joka kasvaa nopeasti, pystyy käyttämään ilmakehän happea ja sen vuoksi sietää huonojakin olosuhteita. Jättikonnamonni voi kasvaa jopa 60 kiloiseksi vonkaleeksi. Tämän lajin erikoisin piirre on kyky liikkua kuivalla maalla ja vaihtaa vesistöä ”kävelemällä”. Vieraisiin ekosysteemeihin päästettynä konnamonnit voivat aiheuttaa suurta tuhoa. Suomen oloissa konnamonni ei selviä talven yli.

Konnamonnit ovat mielenkiintoisia kaloja kalanviljelyn näkökulmasta liittyen erityisesti niiden nopeaan kasvuun ja kykyyn kasvaa suurissa tiheyksissä ja huonohkoissakin vesiolosuhteissa. Konnamonneja onkin sen vuoksi siirretty kalanviljelytarkoituksessa moniin Keski-Euroopan ja Aasian maihin.

Tässä väitöskirjatyössä keskityttiin tutkimaan mahdollisuutta käyttää Nigeriassa helposti ja edullisesti saatavilla olevia kasviraaka-aineita konnamonnille soveltuvina rehukomponentteina. Nigeriassa konnamonni on tärkein kalanviljelylaji, mutta tuotanto ei välttämättä ole kannattavaa, koska maahantuodut kaupalliset rehut ovat kalliita ja toisaalta taas paikalliset itse tehdyt rehut ovat ravitsemuksellisesti heikkoja. Kalliilla rehuilla voidaan saada hyvä kasvu korkeaan hintaan, kun toisaalta halvoilla rehuilla kasvu jää heikoiksi. Molemmissa tapauksissa kalankasvattajan saama tuotto jää alhaiseksi.

Suurin mielenkiinto tässä hankkeessa oli bambarapähkinän käytössä kalanrehun raaka-aineena. Bambarapähkinä on mm. Nigeriassa erittäin edullinen yleisesti käytetty palkokasvi. Muina kasviproteiineina käytettiin seesaminsiemeniä, maissia ja soijaa. Kokeet tehtiin Jyväskylän yliopiston koetiloissa. Kokeet tehtiin siten, että rehujen koostumusta muutettiin kasviraaka-aineiden tai kalajauhon osuuksia muuttamalla. Rehujen vaikutuksia tutkittiin erikokoisilla kaloilla (vastakuoriutuneesta n. 100 g kokoon saakka) mittaamalla kasvua ja rehun hyväksikäyttöä. Rehujen ravintoaineiden sitoutumista kasvuun tutkittiin vakaiden isotooppien avulla, mikä on kalanviljelytutkimuksen näkökulmasta lähes käyttämätön menetelmä. Isotooppien avulla pystyttiin tutkimaan eri rehukomponenttien assimilaatiota.

Tulokset osoittivat, että kalajauho on biologisesti paras raaka-aine konnamonniin kasvatukseen, mutta osa kalajauhosta voidaan korvata kasviraaka-aineilla. Kallis soijajauho voidaan kuitenkin korvata selvästi halvemmalla bambarapähkinällä. Isotooppimittaukset osoittivat, että sekoiterehuissa, joissa on kalajauhoa ja kasviproteiineja, kala assimiloii rehun ravinteista suuren osan juuri kalajauhosta.

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