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Effects of Dietary Lipid Types on Proximate and Fatty Acid Composition of *Clarias gariepinus* in Semi-Intensive System

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Abstract: The effects of different dietary lipids on proximate and fatty acid composition of *Clarias gariepinus* cultured in Semi-intensive system were investigated. Three artificial feed types were formulated with palm oil, beef tallow and combination of palm oil and beef tallow. Commercial feed (Durante) containing fish oil was used as the fourth feed as control. Six weeks old *C. gariepinus* fingerlings of the average weight of 6.2g were stocked in concrete tanks and fed the experimental diets containing different lipid types. The fishes were fed to satiation daily at 8.00 and 16.00 hrs and cultured for 24 weeks and then harvested as table sized fish at average weight of 500g each. Proximate composition and fatty acid profile of the fish was analyzed. The result of the proximate composition showed that crude protein and fat was significantly higher in the palm oil diet than other diets ($P<0.05$) with values of 68.86% and 5.86% for protein and fats for palm oil diet, 68.51% and 5.77%, 67.08% and 5.71% and 66.87% and 5.69% for Durante, beef tallow and combination of palm oil and beef tallow diets for protein and fat respectively. Six fatty acids were detected. They are palmitic acid (28.112%), linoleic acid (24.52%), oleic acid (19.91%), stearic acid (15.57%), linolenic acid (11.09%) and behenic acid (0.998%) in significant decreasing order of abundance. The palm oil diet was observed to be the most utilized in protein and fat synthesis compared to the other diet types.

Key words: *Clarias gariepinus* · Dietary Lipid · Proximate · Fatty Acids Composition

INTRODUCTION

Aquaculture is the farming of aquatic organisms such as fish, crustaceans, mollusk and aquatic plants under controlled conditions. Farming implies some form of intervention in the rearing process to enhance production such as regular stocking, feeding, water quality management, protection from predator among other management practices. In culturing fish in captivity, nothing is more important than good nutrition, because feed represents over 40-50% of the production costs and largely dictates how well the fish will grow if other conditions are right. Ackman [1] reported that the quality of feed provided to the culture fish affects the proximate composition (i.e. protein, lipids, vitamins and mineral content). Okonji and Daniel [2] observed that variation of fatty acids in cultured fish is high under intensive fish culture compared to Semi-intensive culture system. One of the differences between the two culture systems is quantity and quality of artificial feed provided. Feed quality may vary in protein or fats/oil level. Higher quality diet is utilized in higher level of intensification of fish culture and this may affect the quality of fish produced. One marked difference between cultured fishes is the lipid and fatty acid content [3].

Lipids are the generic names assigned to a group of fat soluble compounds found in the tissue of plants and animals. Lipids have many roles which includes; energy supply, structure and precursors to many reactive substances among others. Fatty acids are the main active components of dietary lipids. Lipids are broadly classified as; fats, phospholipids, sphingomyelins, waxes and sterols.

Fats are fatty acid esters of glycerol and are the primary energy depots of animals. Fish have the unique capability of metabolizing these compounds readily and as a result, can exist for long periods of time under conditions of food deprivation. Fats are used for long term energy requirement during periods of extensive exercise or during periods of inadequate food and energy intake. Phospholipids are the esters of fatty acids and phosphaditic acid. These are the main constituent lipids

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of cellular membrane. Waxes are fatty acid esters of long chain alcohols. Sterols are polycyclic, long chain alcohols and function as components of several hormone systems especially in sexual maturation and sex related physiological functions. Sphingomyelins are the fatty acid esters of sphingosine and are present in brain and nerve tissue compounds

Energy is generated by a few basic fuel molecules; glucose, fructose, fatty acids and amino acids [4]. Fatty acids which are found in dietary oils used in making artificial feed are ingested into the fish body as triglycerides. These produce twice the energy as carbohydrates when metabolized [4].

The nutritionally active components of dietary lipids are fatty acids. Fish and mammals appear to be unable to synthesize fatty acids that are unsaturated in the omega-3 and omega-6 positions unless a suitable precursor is supplied in the diet. Thus, the lipid component of the diet must provide an adequate amount of essential fatty acids for growth as well as for required dietary fuel which can be seen in the fatty acid composition of the fish [5]. There are different factors which affects the fatty acid composition of fish [5]. They are; environmental influences and food available, seasonal variations and lipid quality.

Lipid quality in artificial feed may vary with the source of the dietary lipid which also affects the fatty acid composition of the fish. Fatty acid composition in fishes may be seriously affected by the dietary lipid composition of fish fed artificial diet. When the dietary ratio is very high in omega-6 fatty acid supplied by animal lard or vegetable oils, there is a tendency for fish to alter the ratio of poly-unsaturated fatty acids (PUFA) incorporated in favor of omega-3 fatty acid [6]. Fatty acid composition of fish is important as it supplies man with the essential fatty acids (omega-3 and omega-6) and a healthy balance of these fatty acids in human body can greatly reduce the risk of heart related disease and breast cancer. Fish oil is used as a component in aquaculture feed and it is believed to contain balanced fatty acid profile. However, the compition for and high price of fish oil resulted in search for alternative cheaper plant fat/lipid sources [7]. Therefore, the current trend is towards the replacement of fish oil by alternative lipid sources in aquaculture feed for sustainable aquaculture production [8, 9]. However, it is important to know how this will affect the quality of fish produced in terms of fatty acid composition and profile. The effect of animal fats on the growth performance, survival and fatty acid profile had been investigated by researchers and it was observed that total substitution of fish oil with animal fats impairs the nutritional value of the fatty acid composition [10]. Omega-3 poly unsaturated fatty acids content in aquaculture product is a major factor determining their use in fish feed because of their beneficial effects on human health [11].

This study is therefore aimed to determine the fatty acid composition of *Clarias gariepinus* fed with feed containing fats/oil from different sources and how this fatty acid composition affects the ratio of omega-3/omega-6 fatty acids and the unsaturated ratio in the *C. gariepinus*.

MATERIALS AND METHODS

African catfish (*C. gariepinus*) fingerlings of six weeks old and of average weight of 6.2g were purchased from a reputable fish farm in Benin-city. A total of 100 fingerlings of *C. gariepinus* were stocked and cultured for a period of 24 weeks at the University of Benin fish farm under the semi-intensive culture system. The experiment was carried out using a concrete tank measuring 5m by 4m and demarcated into four equal units.

Three experimental diets containing different source of lipids were formulated. The different diets contained palm oil, beef tallow, combination of beef tallow and palm oil (Table 1) and a fourth feed (a commercial diet called Durant) which contained fish oil was used as a reference feed.

The fishes were stocked at the rate of 15 fishes/m^2 and they were fed to satiation twice daily at 8.00 and 16.00 hours. The fish was cultured for 24 weeks using till they are of table size with mean weight of 500g. The table sized fish was then harvested for proximate and fatty acid profile analyses. Good water quality (Table 2) was maintained throughout the culture period through regular flushing of culture water to remove unconsumed feed, fecal waste of fish and other organic and dissolved nutrients.

The whole fish samples were collected from the experimental tank in a fresh state. The fishes were then subsequently killed and oven dried at a temperature of 105°C. The dried whole fish samples were then milled into a fine powder using a hand blender and kept in polythene sachets made air/water tight to prevent microbial spoilage through damping. Thereafter, the samples were taken to the laboratory for proximate analysis. The moisture content of the fish fillets was determined according to Association of Official Analytical Chemists [12]. The samples were dried in a moisture dish in an oven at a temperature of 105°C until

Table 1: Experimental Diets containing Different Types of Lipid

Ingredients (% inclusion)	Diet 1	Diet 2	Diet 3
Fish meal	29.56	29.56	29.56
Ground nut cake	29.56	29.56	29.56
Wheat offal	15.04	15.04	15.04
Lysine	1	1	1
Methionine	0.5	0.5	0.5
Palm oil	9	-	4.5
Beef tallow	-	9	4.5

Table 2: Range of Water quality variables recorded during experimental period

Variable	Minimum	Maximum
Dissolved oxygen (mg/l)	5.8	6.7
pH	6.8	7.5
Ammonia (mg/l)	0.18	0.28
Temperature (°C)	27	29
Total alkalinity(mg/l)	120	128

constant weight was obtained. Ash content of fish samples was determined according to Association of Official Analytical Chemists [12]. Pre -dried samples obtained from the moisture content analysis was ashed in a furnace at 550°C overnight. The crude protein content of the fish samples was determined according to Association of Official Analytical Chemists [12]. One gram of sample was weighed into digestion tubes. Two Kjeldahl CU 3.5 (catalyst salts) were added into each tube. About 20ml of concentrated sulphuric acid (H_2SO_4) was carefully added into the tube and then shaken gently, digestion was then carried out. Digested samples were cooled for 10-20 minutes. Distillation procedure was then performed using the distillation unit and the distillate was titrated with 0.025N sulphuric acid (H_2SO_4) until the end point changes from green to pink. The percentage of protein was then determined using the following equations:

% nitrogen = 0.014 x VD x N x 100 x TV
Weight of sample x AD
% protein = % N x F

where:
VD = Volume of digest
N = Normality of acid
TV = Titre value
AD = Aliquot of digest
F = Conversion factor for nitrogen to protein (6.25)

Crude lipid was obtained by exhaustively extracting 2.0g of each sample in a Soxhlet apparatus using petroleum ether (B.P 40°C-60°C) as the solvent for the lipids. Fatty acids were extracted using a CHC_{13}:MeOH (2:1,V/V) solution in a private laboratory at Ibadan. The samples were centrifuged at 3,000rpm. The supernatants collected added with 0.9% NaCl solution and centrifuged at 3,000rpm. The CHC_{13} phase was evaporated under nitrogen gas and treated with 14% boron trifluoride methanol solution (BF_3-MeOH) for 10 minutes at 100°C. After cooling to room temperature, 1.0ml of water and 2.0ml of pentane were added. The pentane phase was evaporated under nitrogen gas and dissolved using a gas chromatography (GC) (Acme 6000, Young-lin. CO, USA). Nitrogen gas was used for the carrier, the injector and detector temperature were 150°C and 280°C respectively. The temperature gradient of the gas chromatography oven was programmed to be initiated at 180°C for 8 minutes and raised 3°C/minute till it reached a final temperature of 230°C for 15 minutes. Individual fatty methyl esters (FAME) were quantified as a percentage of total FAME analyzed.

Each treatment (sample) was replicated thrice through the analyses and treated as complete randomized design. Data collected was analyzed using Genstart Statistical Package Version 8. Results of the analysis were tested using table of analysis of variance. Means were separated using Duncan's multiple range test. All tests were carried out at 5% probability level.

RESULTS

The result of proximate composition of *C. gariepinus* fed different feed types is shown in Table 3. There was a significant difference in Crude protein, Fat, Ash, CHO, Fiber and Moisture content in the *C. gariepinus* fed diets with the different dietary lipid over time ($P<0.05$). Fish fed palm oil diet had higher Crude protein content of 68.86% than the fishes fed other feed types. This is followed by fishes fed Durante feed, beef tallow diet and the least was palm oil and beef tallow combination diet with values of 68.51%, 67.08% and 66.87% respectively ($P<0.05$).

Fish fed palm oil diet had significantly higher Fat content of 5.86%, than other fishes with fishes fed Durante feed, beef tallow diet, palm oil and beef tallow combination having 5.77%, 5.72% and 5.69% fat respectively ($P<0.05$).

Mean feed type shows that the fishes fed Durante feed had higher Ash content of 6.13% closely followed by the fishes fed palm oil diet with 6.09% and palm oil and beef tallow combination diet with 6.063% and the beef tallow diet with 5.96%. There was no significant difference ($P>0.05$) between palm oil diet and palm oil and beef tallow combination diet.

Table 3: Proximate Composition of *C. gariepinus* Fed Feed Containing Different Types of Dietary Lipid

Treatment (Diet Types)	ASH (%)	CHO (%)	FAT (%)	FIBRE (%)	MOISTURE CONTENT (%)	PROTEIN (%)
Beef tallow diet	5.9575^{c}	11.0950^{a}	5.7150^{c}	0.3100^{c}	10.0150^{c}	67.0800^{c}
Durante feed	6.1300^{a}	9.1400^{c}	5.7700^{b}	0.3400^{b}	10.2775^{b}	68.5100^{b}
Palm oil diet	6.0900^{b}	9.0025^{d}	5.8600^{a}	0.3650^{a}	10.0125^{c}	68.8575^{a}
Palm oil/ beef tallow Combination diet	6.0625^{b}	10.800^{b}	5.6875^{d}	0.3075^{c}	10.4625^{a}	66.8725^{d}

N:B Means with different alphabetic subscript are significantly different at 5% probability levels. Vertical comparison only

Table 4: Mean Proportions of Different Types of Fatty Acid Fed Different Dietary Lipids

		Proportion of Fatty Acid Types (%)					
	Treatment	Behenic	Linoleic	Linolenic	Oleic	Palmitic	Stearic
	Beef tallow diet	2.3750	26.4350	12.5500	16.9150	26.9450	15.7450
	Durante feed	3.2850	26.1750	12.4750	15.7650	26.9250	16.3550
	Palm oil diet	2.6750	26.3450	12.4150	16.2250	26.5350	15.7850
	Palm oil + beef tallow diet	3.1150	26.2250	12.7750	16.8850	25.9550	16.0450
	Beef tallow diet	0.3250	23.5450	10.3100	21.3600	28.6800	15.4700
	Durante feed	0.9050	23.4650	10.2300	21.5400	28.4750	15.4050
	Palm oil diet	0.6000	23.6550	10.3550	21.2600	28.3400	15.7850
	Palm oil + beef tallow diet	0.0150	23.6500	10.4150	21.7550	28.2850	15.8750
	Beef tallow diet	0.2950	23.5850	10.4250	21.4750	28.7250	15.4850
	Durante feed	0.4250	23.5650	10.3550	21.4750	28.6850	15.4850
	Palm oil diet	0.3150	23.6250	10.3550	21.3850	28.6650	15.5850
	Palm oil + beef tallow diet	0.3250	23.5950	10.4450	21.4450	28.6650	15.5350
Mean feed type	Beef tallow diet	0.9983^{f}	24.5217^{b}	11.0950^{e}	19.9167^{c}	28.1167^{a}	15.5667^{d}
	Durante feed	1.5383^{f}	24.4017^{b}	11.0200^{e}	19.5953^{c}	28.0283^{a}	15.7483^{d}
	Palm oil diet	1.1517^{f}	24.4900^{b}	11.2117^{e}	20.0283^{c}	27.6350^{a}	15.8183^{d}
	Palm oil + beef tallow diet	1.1967^{f}	24.5417^{b}	11.0417^{e}	19.6233^{c}	27.8467^{a}	15.7183^{d}

N:B Means with different subscript are significantly different at 5% probability levels. Horizontal comparison for each feed type

CHO content is higher in the fishes fed beef tallow diet at 11.095%, followed by the fishes fed palm oil and beef tallow combination diet, Durante feed and palm oil diet with values of 10.80%, 9.14% and 9.00% respectively (P<0.05).

Fishes fed palm oil diet also had higher fiber content than in the fishes fed the other feed types at 0.37%, Durante feed 0.34%, beef tallow diet 0.31% and the palm oil and beef tallow combination diet 0.31%. There was no significant difference (P<0.05) between the beef tallow and the palm oil and beef tallow combination diet.

Moisture content is higher in the fishes fed Palm oil and beef tallow combination diet with 10.46% moisture. This was followed by the fishes fed Durante feed with 10.28% moisture, fishes fed beef tallow diet with 10.02% moisture and palm oil diet with 10.01% moisture. There is no significant difference (P<0.05) between the moisture content of the fishes fed beef tallow diet and the fishes fed palm oil diet.

The results of the proportions of different types of fatty acids in *C. gariepinus* fed different dietary lipids are shown in Table 4.

Table 4 shows that the same fatty acid types were identified in all the fishes fed different diet types. There was a significant difference (P<0.05) in the proportion of various types of fatty acids. Palmitic acid had the highest concentration among the fatty acid types (p< 0.05). This was followed by lioleic, oleic, strearic and behenic acids in significant decreasing order of abundance (p<0.05). This distribution is similar in all the diets. Two polyunsaturated fatty acids were detected. These are linoleic (w-6) and linolenic (w-3) with w-3/w6 ratio of 1: 2

DISCUSSION

Result from Table 3 indicated variation in the proximate composition of *C. gariepinus* fed different feed types. Variation in the proximate composition of *C. gariepinus* may have resulted from the different lipid types in the feed. The proportion of fish in the food composition of adult human specimen reaches 80% and as such a major source human dietary needs. The protein and fat contents of the fish carcass composition showed significant difference between the different feed types with palm oil having higher protein and crude fat. Similar results were also found for *Oreochromis niloticus* [13], European sea bass [14, 15]. and Rainbow trout [16]. In all the studies, the palm oil diet appeared to be better utilized for protein and fat synthesis. *C. gariepinus* has the ability to utilize palm oil for energy generation and protein formation. This is in line with Bruton [17] who suggested the use of plant lipid sources for fish feed formulation. The palm oil diet appears to be better utilized for protein synthesis than that of animal lipids. The results in Table 3 also indicated that carbohydrate levels of 11.1%, 9.14%, 9.00% and 10.8% for fishes fed beef tallow diet, Durante feed, Palm oil diet and combination of palm oil and beef tallow diet. This result indicated lower carbohydrate for palm oil diet as observed by Mollah and Hossain [18]. This may be attributed to the higher utilization of palm oil for protein and fat synthesis. The fishes showed relatively low moisture content, with the moisture content not exceeding 10.5% in fishes fed palm oil and beef tallow combination diet which showed the highest moisture content. This is in line with the work of Steven and Helfrich [19], who observed that moisture content should not exceed 10%-11%. This also corresponds with the work of Peter and Ann [20], who observed that the low value of moisture is because the fishes were dried before analysis and the high content of moisture causes spoilage organisms to thrive thus causing spoilage. The analyzed ash content of the fish samples for the different feed types showed that the fishes fed Durante feed had the highest at 6.13% and those fed beef tallow diet had the lowest at 5.96%. This corresponds with the observation made by Steven and Helfrich [19], which showed that a complete diet has an ash level of <8.5% when fishes are reared in semi-intensive system. The observed range of ash content indicated that the different diet types are a good source of minerals such as Calcium, potassium, zinc, iron and magnesium. Fiber content was highest in the fishes fed palm oil diet with 0.37% and lowest in fishes fed beef tallow diet (0.31%) and palm oil and beef tallow combination diet (0.31%). This is in line with the work of Montero *et al.* [14], who found out that fishes do not need large amount of fiber in their feed and this therefore translates into low fiber content in the fish flesh. Also, De Silva [21] observed that the role of fiber in the fish diet is basically to aid in digestion.

Turchini *et al*. [8] observed that the different lipids present in the different feed type fed to fish could be the main reason behind the different fat content observed in their carcass. This experiment showed that the fish fed palm oil diet had higher fat content of 5.86% and the fish fed palm oil and beef tallow combination diet having the lowest (5.69%). This is in line with findings of Turchini *et al*. [8] which observed that 5%-20% of lipid in most fresh water diets gives optimal growth rates without producing an excessively fatty carcass. Ozugul and Ozugul [22] also observed that fatty fish usually contains a minimum of 5%-8% fat in edible tissue. Although, New [23] reported that some salmonid feed have dietary lipid levels as high as 35%. Dietary lipids provide essential fatty acids (Polyunsaturated fatty acids, PUFAs) that fish like all animals cannot synthesize but require for the maintenance of cellular function. According to New [24] and Tacon [25] plant oils are generally rich sources of linoleic series fatty acid (n-6) and with the exception of linseed, conopher seed and hempseed oils that contain little or no linolenic series fatty acid (n-3). Tacon [25] observed that linolenic series of fatty acids are found in terrestrial animal fats (like beef tallow) only in trace amounts and are common only in marine oils. Just as in other fishes, *C. gariepinus* produces high levels of omega-3 polyunsaturated fatty acids in the meat [26, 27]. According to Oresegun *et al.* [28], *C. gariepinus* is a commercially important specie in Nigeria and is one of the most cultured specie and source of essential fatty acid such as linolenic (C18:3) and linoleic (C18:2). Table 3 indicates the six fatty acid types in *C. gariepinus* fed different feed types. These includes Behenic acid (C22:0), Linoleic acid (C18:2), Linolenic acid (C18:3), Oleic acid (C18:1), Palmitic acid (C16:0) and Stearic acid (C18:0).

Palmitic acid (C16:0) was the predominant saturated fatty acid in the *C. gariepinus* fed different feed types. Ackman [1] observed that palmitic acid was a key metabolite in fish which level was not influenced by diet. The polyunsaturated fatty acid found in this study was similar to those of other species. Similar studies on tropical [13] and temperate [29] freshwater fishes indicated the dominance of these fatty acids in the tissue lipids of fish. The fatty acid found in the different feed types in this study was characterized by high linolenic (n-3) fatty acids. The results agree with those obtained from other studies conducted on freshwater fish species.

Kris-Etherton *et al.* [30] observed that at sufficiently high intakes, long chain omega-3 polyunsaturated fatty acids as found in oily fish and fish oil have been shown to reduce inflammation and may help prevent chronic diseases. From the study of the different feed types, it was observed that fishes fed palm oil and beef tallow combination diet had a higher linoleic (C18:2) fatty acid level than those fed the other three diets. However, there was no significant difference between the linoleic (C18:2) fatty acid level between the different diets. Fishes fed palm oil diet had the highest linolenic (C18:3) fatty acid level while those fed Durante feed had the lowest. The results of this study indicate an omega-6 to omega-3 ratio of 2:1 for each of the diet types. Harper and Jacobson [31] observed that a lower ratio of omega-6 to omega-3 fatty acids is more desirable in reducing the risk of many chronic diseases of high prevalence in Western societies as well as in the developing countries. Thus, indicating that of the different feed types the ratio from this study has a desirable n-6/n-3 fatty acid ratio which is suitable for consumption. Thus in terms of fatty acid composition and quality, the plant lipid (palm oil) source can replace the expensive fish oil which is in short supply without compromising quality and quantity.

CONCLUSION

The study revealed that all the four feed types are good source of essential fatty acid and contain a fairly good proportion of omega-6 to omega-3 fatty acid ratio which is required for good health. The study also shows that palm oil inclusion in fish feed, a cheaper and available source of lipid is a better source of lipid that may guarantee high protein and fat composition of culture fish. It also met the omega-6 to omega-3 ratio, provided the necessary fatty acid needed and have a good proximate composition of feed nutrients especially protein. The demand for fish oil is very high; thereby making it expensive, aquaculturists should concentrate on the use of palm oil as lipid source in fish feed as it generates higher fat and protein level. However, the choice of oil should be based on availability and cost in a particular location. More attention should also be given to the use of other plant lipid sources to reduce cost of feed.

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