Feed utilization and Haematological parameters of *Clarias gariepinus* post fingerlings fed varying levels of maize offal diets.

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Abstract

An eight weeks feeding trials was conducted at the wet laboratory of Cross River University of Technology to evaluate feed utilization and haematological parameters of *C. gariepinus* fed varying levels of maize offal diets. A total of 300 hundred apparently healthy post fingerlings with the mean length of 10.06±3.21cm and weight of 8.0±2.08g were allocated to five varying treatments of maize offal diets at 0%, 25%, 50%, 75% and 100% substitution level representing diets A, B, C, D and E respectively. Twenty post fingerlings were stocked in each tank of 50 litres of water with each treatment replicated thrice. The weight and quantity of feed intake were recorded fortnightly. At the end of 8 weeks, blood samples were collected for analysis of some haematological parameters. The result revealed that fish fed 75% level had significantly higher (P<0.05) mean protein efficiency ratio (PER) and feed conversion ratio (FCR) than 100% and control (0%) respectively. It also shows that the value of the packed cell volume (PCV) was significantly (P<0.05) higher in fish fed 75% maize offal diet than those of 100% and control. Similar results was recorded for red blood cell (RBC), white blood cell (WBC) and platelet (Plt) whereas haemoglobin (Hb) and mean cell haemoglobin concentration (MCHC) did not show any significant different (P>0.05). All the differential cell counts increase with increasing level of maize offal diets (P<0.05). However all the haematological parameters recorded were within the range recommended for apparently healthy fish. At the substitution level of 75% *C. gariepinus* produced the best food utilization parameters and haematological parameters than other level. The study revealed that maize offal had no negative impact on the health status of *C. gariepinus* with a better feed utilization, PC, Hb and differential counts when incorporated up to 75%. Therefore it can be concluded that maize offal can be used replace cereals up to 75% in the nutrition of *C. gariepinus* to enhance growth performance and fish health.

Keywords

Maize offal, Feed utilization, Haematological parameters and *Clarias gariepinus*.

Introduction

Fish is often the cheapest source of animal protein and is, therefore, important in the diets of the lowest income group (Allison, 2001). One of the proposed solutions to the crisis in capture fisheries which contributes 80% of total fish production (FAO, 1998) has been to resort to fish farming. The use of wild-caught fish as fishmeal in fish diets puts further pressure on stocks (Naylor et al., 2000). To meet the protein demand in developing countries where animal protein intake is also grossly inadequate and relatively expensive, intensive research effort is geared towards finding alternative sources of protein and energy from underutilized grain legume seeds (Adaparusi, 1994; Osuigwe, 1999). However, feed being a major inputs in aquaculture production, high cost of fish feed has caused a lot of problem in aquaculture sector, which has actually hindering aquaculture development in Nigeria, accounting for at least 60% of the production cost (Gabriel et al., 2007). Expensive feeds has actually reduced the profitability of fish farming thereby limiting the expansion of farms and reducing the yield in terms of quantity and quality (Adikwu, 1992). This has brought about the search for local protein feed stuffs that are cheap and high in quality as alternative protein feed for *C. gariepinus* quality. In Nigeria the most popularly incorporated cereal grain in feed formulation is maize where it supplies more than half of the metabolizable Energy (ME) requirement of fish for growth and development, (Ravindra and Ravindra 1988, Durunna et al; 2000). The incessant high cost of maize as main energy source for livestock and fish feeds being a staple food for Nigerians and Agro allied companies including brewing industry cannot be over emphasized. Many attempts have been made to solving this problem through the use of some non – conventional energy source in feed formulation as maize offal (Vantsava et al., 2008). The African Catfish *C. gariepinus* is widely considered to be one of the most important tropical catfish species for aquaculture. It has a Pan African distribution, from Nile to West Africa and from Algeria to South Africa. The African catfish has a high growth rate; it is very resistant to handling stress and is very well appreciated in a wide number of African countries including Nigeria. Maize offal is a carbohydrate and it remains the cheapest source of dietary energy in domestic animals including fish. Carbohydrates are important non – protein energy sources for fish and should be included in their diets at appropriate levels which maximize the use of dietary protein for growth. Feed utilization has been widely used as indices to determine the effectiveness and efficiency of any given diet. Olaniyi et al (2013).Presence of certain limiting factors in plant ingredients such as high crude fibre content and anti-nutritional factors have been demonstrated as the major factor affecting nutrient utilization (Alegbeleye et al., 2001; Nwanna et al., 2008). Excessive consumption of plant protein sources by fish could cause slower growth rates and poor performance which may result in mortalities if condition persists (Makkar et al., 2001). The importance of feed intake by fish as a determinant of fish performance has been strongly emphasized (Preston et al., 1987).
Faturoti, 1989; Pillay, 1990) while other studies Anderson et al., (1984) and Gatha et al.(1993) pointed out the possibility of protein sparing effects by other nutrients in a feed, that is, as more energy was supplied for metabolism through other nutrients, more protein intake is available for fish growth and tissue development. Haematological characteristics of most fish have been studied with the aim of establishing normal value range and deviation from it may indicate a disturbance in the physiological process (Rainzapaiva et al., 2000). Environmental and physiological factors are known to influence fish haematology, include stress due to capturing, transportation, sampling, age, sex and diet (Bello et al., 2013). Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Oyawoye and Ogunkunle, 1998). According to Ayoola et al (2013) platelet of C. gariepinus fed different level of probiotic for 60days increase compared with control diets.

According to Wilson (1994) certain fish species exhibit reduced growth rates when fed carbohydrates free diets. However Peragon et al: (1999) reported that carbohydrate affects nutrient utilization in muscle of Rainbow Trout (Oncorhynchus mykiss). Excessive dietary carbohydrate in fish diet may also lead to fat deposition by stimulating the activities of lipogenic enzymes. Orire and Sadiku et al (2013) reported that growth performance and feed utilization of C. gariepinus fed corn fibre did not show any significance difference. They further stated that FCR has no significant difference whereas PER was significantly higher in treatment 15:25 and 20:20 of corn / protein ration. Protein efficiency ratio (PER) was highest in fish fed with 10% M. oleifera leaf diet, which was not statistically significant from value of 0% M. oleifera leaf diet in fish fed between fingerling and juvenile stage (Bello and Nze 2013). Olaniyi et al (2013) reported a higher FCR and PER values in C. gariepinus fed varying levels of M. oleifera leaf meal. They stated that the higher the protein intake the higher the utilized nutrients. Growth and feed utilization decrease from fish feed diet above 75% dry Moringa seed meal inclusion level (Obasa et al 2013). According to Falayi et al (2013) the best growth and feed utilization parameters were obtained in control diet with 50% Tilapia fish meal and the lowest values in diets 1 with 50% seaweal seed cake on C. gariepinus. Agbebi et al (2013) reported that 30% garlic inclusion ratio in a compounded feed helps in feed utilization and growth performance with no negative effect to the tissues C. gariepinus . However Bello and Nze (2013) reported that growth and Nutrient utilization by C. gariepinus fingerlings decreased as M. oleifera leaf meal increased in the diet.

Carbohydrate utilization is much more variable and probably is related to natural feeding habits and incorporation of this nutrient may add beneficial effects to the quality and economy of Aquaculture production.

Since maize offal is cheap and readily available and less competed by human and livestock, it therefore remains the cheapest sources of energy supplement. However several researches have been on that of protein, hence the need for this research. The aims of this research is therefore to determine the effect of maize offal formulated diets on feed utilization and haematological parameters of C. gariepinus.

Materials and Methods

Experimental Sites

The research was conducted at the laboratory of the Department of Fisheries and Aquatic Science, Cross River University of technology, (CRUTECH) Obubra.

Experimental Fish

Three hundred and fifty apparently healthy post fingerlings of C. gariepinus were procured from University of Calabar (Unical) Fish farm in Cross River State Nigeria. They were put in 20 litres jerry cans and transported by Car to the wet laboratory of Fisheries and Aquatic Science.

They were batch weighed and put in aquaria before acclimated for two weeks before the commencement of the research. During the period of acclimation the fish were fed with vital fish feed with a crude protein of 40% twice daily for two weeks.

Source of Maize Offal and Formulation of Experimental Diet

Maize offal was obtained from Apiapum Market Adun – Obubra and was properly dried and grinded before use in the formulation process. Five (5) different diets were prepared using Trial and Error method of fish formulation to obtain 40% crude protein. The maize offal was incorporated into each of the diet at 0% (control), 25%, 50%, 75% and 100% to replace with equal weight of maize as shown in table 3.1. The experimental diets were subjected to proximate analysis following the procedures of AOAC (2000).

<table>
<thead>
<tr>
<th>Ingredients (100g)</th>
<th>Diets (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal (65%cp)</td>
<td>A (0)</td>
</tr>
<tr>
<td></td>
<td>32.00</td>
</tr>
<tr>
<td>Soybean meal (45%cp)</td>
<td>16.00</td>
</tr>
<tr>
<td>Blood meal (80%cp)</td>
<td>12.00</td>
</tr>
<tr>
<td>Maize (10%cp)</td>
<td>20.00</td>
</tr>
<tr>
<td>Maize offal (10%cp)</td>
<td>0.00</td>
</tr>
<tr>
<td>Palm Kernel cake (7%cp)</td>
<td>10.00</td>
</tr>
<tr>
<td>Fish oil</td>
<td>3.00</td>
</tr>
<tr>
<td>Palm oil</td>
<td>3.00</td>
</tr>
<tr>
<td>Vit/min premix</td>
<td>2.00</td>
</tr>
<tr>
<td>Binder</td>
<td>2.00</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
<tr>
<td>Crude protein level (%)</td>
<td>40.30</td>
</tr>
</tbody>
</table>

Table 3.1 Percentage Composition of Experimental Diets.

Experimental Design and Procedure

Three hundred (300) post fingerlings of C. gariepinus with mean length of 10.06± 3.21cm and body weight of 8.0±2.08 g were randomly distributed to 15 aquaria, after acclimation according to Okere ef al. (2001). Fifteen rectangular plastic tanks were washed and disinfected before randomly allocating the fish into the five treatments diets A, B, C, D, and E in triplicates. The fish were starved for 24hours prior to the commencement of the experiment. This practice was to eliminate variations in weight due to residue food in the gut and also to diets while at the same time to increase the appetite of the fish. The initial body weights were taken using mettle top loading balance for each tank. Feeding was carried out twice daily 8:00 am and 5:00pm at 5% body weight. Subsequently, total weight, total length and quantity of food consumed were taken fortnightly.

Determination of Nutrient Utilization

Protein Efficiency Ratio (PER): The PER was determined according to Wilson (1989) It was calculated from the relationship between the increment in the weight of fish (i.e. weight of fish and the protein consumed). It is represented by the formula

\[
\text{PER} = \frac{\text{Weight gain (g)}}{\text{Protein intake}}
\]

Feed Conversion Ratio (FCR): The FCR was determined from the relationship between feed intakes over body weight and is calculated from the formula

\[
\text{FCR} = \frac{\text{Feed intake}}{\text{Fish weight gain}}
\]
Blood Collection and Haematological Analysis

Blood samples were collected at the 8th week of the experiments following the procedure of Schmitt et al. (1999). Blood of 2ml were collected from caudal peduncle with the aid of a 2ml sterile plastic syringe fitted with 0.8 x 38mm hypodermic needles. The blood was collected in triplicates into sample bottles containing ethylene diamine tetra acid (EDTA) as anti-coagulant. The blood was rocked gently in the bottle to allow thorough mixing of its content. The samples were preserved in a cooler containing ice block and thereafter transported to University of Calabar Teaching Hospital (UCTH), haematology Department for analysis within six hours after collection. The haematological parameter determined were Red blood cell (RBC), White blood cell (WBC), Platelet (Pt), Pack cell volume (PCV), Haemoglobin (Hb) and Differential counts. The mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were calculated from the data using standard formulae (Lee et al; 1998). The direct measurements of erythrocyte value (Packed cell volume PCV, Haemoglobin Hb, and Red blood cell RBC), absolute erythrocyte indices (MCH, MCV and MCHC) were calculated. The platelets, white blood cell and differential count (neutrophils and lymphocytes) were analysed as described by Davie and Lewis (2001); Ochei and Kohkater (2003).

PCV (%) = \( \frac{\text{Packed RBC column height} \times 10^{6}}{\text{Total blood column height} \times 10^{4}} \times 100 \)

Red blood cell count = \( \frac{N \times D \times F \times 10^{6}}{A \times D} \times (100,000) \)

Where: N = number of cells counted
DF = dilution factor (200)
A = area of chamber (0.2mm²)
D = depth of chamber (0.1 mm)
10⁶ = convert to cells per litre x 10³/l

White blood Count = \( \frac{N \times D \times F \times 10^{6}}{A \times D} \)

Mean Cell Haemoglobin = Haemoglobin (g/l) / RBC (10¹²/l)

Mean Cell Haemoglobin Concentration (g/100ml = Haemoglobin (g/l) x 100 / PCV %)

Mean Cell Volume (fl) = PCV % \times 100 / RBC (10¹²/l)

Statistical Analysis

The data collected were subjected to one way analysis of variance (ANOVA) using SPSS version 20. Comparison among diet means for each parameters were carried out using Turkey’s honest significant different (HSD) at significant level of 0.05.

Results

Nutrient Utilization

The result of the nutrient utilization by C. gariepinus is shown in table. The PER did not show any significant effect with increase dietary maize offal except at 75% substitution level which was significantly higher (P<0.05) than in fish fed control and 100% diets.

The parameter of FCR showed that fish fed with 75% maize offal had a significant (P<0.05) increase value than those fed 100% and control diets. The highest PER value of 4.73 and least of 2.13 were recorded in fish fed 75% and control (0.0%) diets respectively. Similarly the highest FCR value of 4.90 and lowest of 2.11 were obtained in fish fed 75% and 100% maize offal diets respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A (0)</th>
<th>B (25)</th>
<th>C (50)</th>
<th>D (75)</th>
<th>E (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Efficiency Ratio (PER)</td>
<td>2.13±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.42±0.87&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.30±2.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.73±2.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.96±2.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feed Conversion Ratio (FCR)</td>
<td>2.61±0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.26±3.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.02±1.26&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.90±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.11±1.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with the same superscript under the same rows are not significant (P > 0.05)

Table 1: Nutrient Utilization of C. gariepinus Post Fingerling, Fed Dietary Levels of Maize Offal for 8 Weeks.

Haematological Parameters of C. gariepinus Feed Different Levels of Maize Offal Diets

The result of the haematological parameters of C. gariepinus is shown in table (4.2a and b). The result revealed that the value of the PCV was significantly (P<0.05) higher in fish feed 75% maize Offal diet than to those of 100% and control. Similar results were recorded for RBC, WBC and Pt whereas Hb and MCHC did not show any significant different (P>0.05). The values of MCV and MCH decrease significantly (P<0.05) with increasing levels of maize offal after 8 weeks of feeding trial. The highest value of 8.61(g/l) and lowest of 6.80(g/l) for Hb was recorded in fish fed with 75% and 100% respectively.

The result obtained for RBC revealed that there was an increase in RBC in fish feed with increase percentage maize offal diet except at 100%. For instance the highest value of 7.78x1012 cells/L and the lowest values of 4.55x10¹² cells/L of RBC in fish fed with 75% and control was recorded respectively. However, at 100% level the RBC decreased with the value of 5.65x10¹² cells/L. Also decreasing were the MCV 72.29fl to 48.44fl and MCH 16.22 pg to 11.22 pg in fish feed control (0.0%) and 75% maize offal diet respectively.

The result of the deferential counts shows that there was significant different (P<0.05) in fish fed with 75% maize offal diet and those of the control. The means values of all the parameter studied were higher in fish fed with 75% maize offal than those of 100% and control except for eosinophil which was not significant (P>0.05) from those fed 100% maize offal diet.

Table 4.2(b): Mean values of the Haemalogical parameters of C. gariepinus Fed Different levels of Maize Offal Diet for 8 Weeks.
The means with the same superscript under the same parameter are not significant (P>0.05)

**Table 4.2(b):** Mean values of different white blood count of *C. gariepinus* fed different levels of maize offal diets.

### Discussion

#### Nutrient Utilization

Maize offal is a cheap source of dietary feed for fish (Shiau and Linn, 2001). According to Fagbenro et al. (2003) and NRC (1993), incorporation of carbohydrate are beneficial to the pelleting quality of the diet and fish growth. However, excessive dietary carbohydrate in fish diet may lead to fat deposition (Likimani and Wilson, 1982). The result on fed utilization in this study shows an increase as the dietary maize offal increased to 75% substitution level. This observation supports the findings of previous studies. Orire and Sadiku (2013) showed that three levels of carbohydrate 5.10 and 20% of three carbohydrates sources corn fibre, corn starch and glucose had a significant increase in growth and nutrient utilization. According to Orire and Sadiku (2013) the corn starch based diets at higher inclusion level (15.25), performed better than other carbohydrate sources. The mean values of PER in control and 100% dietary level were lower than other levels in this study. The result seems to have direct link with palatability of the diet which causes reduced feed intake. Similar observation was reported by Bello et al. (2013) on *C. gariepinus* fed different levels of *M. oleifera* leave diets. The importance of feed intake by fish as determinants of fish performance has been strongly emphasized (Prston et al. 1984; Faturoti, 1989). This could also be that at the control diet there was no enough carbohydrate and the protein was converted to energy rather than for growth whereas at 100% substitution there was no sufficient protein to enhance proper growth. At 75% with the highest values of PER 4.73 and FCR 4.90 is an indication that there was sufficient energy supplied for metabolism and the available protein use for fish growth and tissue development. Fabgero, et al. (1993) reported that carbohydrate levels in *C. gariepinus* diet often can be substantial and reportedly range from 15-35%. In this study substitution range of 75% gave a maximum fed utilization parameter indicating a much higher inclusion levels than those earlier reported by, Orire and Sadiku, (2013). The inclusion of maize offal up to 75% level in the diet of *C. gariepinus* in this study without any negative effects on feed utilization and growth confirmed the ability of *C. gariepinus* to utilized carbohydrate levels beyond a certain point. Excessive carbohydrates have been reported to depress feed efficiency, growth and even cause eventual mortality according to Orire and Sadiku (2013).

#### Haematological Parameters

Haematological parameters have been widely used in clinical diagnoses of disease and pathological of human and domestic animals. The applications of haematological techniques have proved valuable for fishery biologist in assessing the health of fish and monitoring stress responses according to Osuigwe et al. (2005). Haematological parameters of fish are affected by a range of factor which includes size, age, physiological status, environmental conditions and dietary regime.

The haematological results of the present study shows that all the parameters were affected by dietary regime except for Hb and MCHC. However fish fed with 75% maize offal tend to have higher values across the parameters studies, although in most cases not significant.

All these were within the recommended physiological ranges reported for *C. gariepinus* (Adam and Agab, 2008). Information on the effect of maize offal (carbohydrate) on haematology of fish is limited. However several works have been reported on various protein supplements on *C. gariepinus*. The result of this study on haematology disagrees with those of Bello et al. (2013); Osuigwe et al. (2005) who both reported a decrease in some haematological parameter when *C. gariepinus* was fed different levels of *M. oleifera* and *Canavalia enciformis* (Jackbean) diets respectively. This reduction was attributed to the presence of anti-nutritional factors in the feed. This implies that maize offal may not have contained some of those anti nutrients that would have caused negative effects on the haematology of *C. gariepinus*. According to Oyawoye and Ogunkune (1998) reduction in PCV is an indication of presence of toxic factor which has adverse effects on blood formation. The observed reduction in haematological parameters in *C. gariepinus* fed 100% maize offal diet in this study conforms to reports of Tacon, (1992) and that nutritionally deficient diets cause decrease in haemoglobin, PCV and RBC (Adam and Agab, 2008). Physiologically haemoglobin in crucial to the survival of fish being directly related to the Oxygen binding capacity of blood. However the reduction between 8.69 to 6.80g/ml at the 75% to 100% maize offal levels respectively may not have had deleterious effects on *C. gariepinus* given that the values are within the normal range recorded for African Catfish (Musa and Omorieg, 1999). The white blood cells and its differential parameters are the defence cells of the body. Douglass and James (2010) demonstrated that the amount has implication in immune responses and the ability of the animal to fight infection. In this study white blood cell, lymphocytes and other differential parameters, showed increase as the level of maize offal in the diet increase except at the 100% substitution level. According to Oyawoye and Ogunkune 1998 high values of WBC and lymphocytes are usually associated with microbial infection or the presents of foreign body or antigen in the circulating system. Bello et al (2013) reported that high values of white blood cell and lymphocytes recorded in *C. gariepinus* fed Oeleifera diet was an indication of feed toxicity. Since the values in this study was within the normal range the increase antigen and antibody was mere preparing the body against any foreign invasion into the body.

This study therefore highlights the fact that maize offal can be substituted up to 75% on diet of *C. gariepinus* with maximum growth and feed utilization without any adverse effects on the haematological parameter.

### Conclusion

The result of the 8 weeks feeding trial of *C. gariepinus* with maize offal diets shows that maize offal could be used in aquaculture for fish production. However feed utilization and haematological parameter could be improve in fish by incorporating maize offal at 75%. It can also be deduced that 100% level reduce feed utilization and some blood parameter meaning that there was no enough protein in the diet to build up for this cells and also reduces palatability of the feed.

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