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Growth and Haematological Effects of Replacing Maize with Feed Wastes on the Juvenile *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

Viability and profitability of fish farming enterprise depends largely on the total cost of fish feeds. A feeding trial to develop practical and cost effective diets was conducted to determine the growth and haematological effects of feed waste meals on *Clarias gariepinus* juveniles. Total of 120 juveniles (11.5±0.8 g) were randomly divided into four treatments each with two replicates of fifteen juveniles and distributed into happa (0.7 m^3). The happas were suspended to 3/4 of their volume using kuralon ropes tied round the bamboo poles across the earthen pond. The juveniles were fed at 3% body weight per day for 8 weeks. Four diets containing 40% crude protein were formulated in which maize was replaced with biscuit waste, Indomine® (noodles) and Gala® (sausage roll) waste meals at treatment diet 1 (Maize), treatment diet 2 (biscuit waste), treatment diet 3 (Indomine®) and treatment diet 4 (Gala®) levels. There were no significant differences ($p>0.05$) in the Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR) in treatment 1 (control) and other treatments in the experiment. It was recorded at the end of the experiment that biscuit, Indomine® and Gala® wastes has equal potentials in replacing maize as an energy supplement when incorporated to the *Clarias gariepinus* feed. Cost analysis reveals that the use of any of these wastes in replacing maize could reduce total feed cost per kg by at least 30%.

Key words: Growth, haematology, maize, feed wastes, *Clarias gariepinus*

INTRODUCTION

Fresh fish contains high quality of proteins that can be used to maintain an active metabolism. Fish contains low fat and low cholesterol level which makes the muscle a good antidote for fat related diseases. Tilapia and catfish are the major fish species cultured in the Nigerian aquaculture sector. The sector is currently faced with the challenge of inadequate and prohibitive cost of quality fish feeds. Therefore, attempt to develop practical diets for farmed fish is necessary (Ayoola, 2010; Fagbenro *et al*., 2003; Omitoyin, 2005). The cost quality and quantity of the feed forms the major part of cost of production and performance of fish. The cost of maize is relatively high compared to other sources as a result of its human competing uses (especially in developing African countries). This has made it necessary to evaluate other ingredients to replace maize which is the major source of energy with cheaper carbohydrates (Olurin *et al*., 2006), hence there is need to look for alternative sources.

Wastes from industries and domestics can be a relief in which many of these are cheap and readily available in very large quantity. They are usually agro Industrial by-products. Some are

animal or plant based for example noodles, sausage, biscuit, yam and cassava peel. Generally these materials are relatively cheap and available throughout the year. However, the potency of a feed is a preferred determinant and not the cost (Falaye, 1992). Hence, a balanced diet is therefore required for aquaculture to be profitable.

Biscuit waste, Indomine® (noodles) and Gala® (sausage roll) waste are industrial waste products found in substantial quantities at different industrial areas in Nigeria. The costs of these industrial wastes are relatively low compared to that of maize as they are categorized as waste products. Biscuit Waste, Indomine® (noodles) and Gala® (sausage roll) waste have no anti-nutritional factor and could make a good replacement for maize and other cereals Longe (1986). This study was conducted to determine the effect of feeding catfish with biscuit, Indomine® (noodles) and Gala® (sausage roll) wastes as replacement for maize fraction of the diet to enhance growth performance and cost maximization on the feeds.

MATERIALS AND METHODS

Experimental site and set-up: The fish culture experiment was carried out in net happas rearing medium suspended to 3/4 of their volume in water and stabilized in 12×12×1.5 m earthen pond using kuralon twine tied horizontally to poles and dig into the pond bottom. The juvenile *Clarias gariepinus* were obtained from a private farm and acclimatized for one week. During the period of acclimatization, the fish were fed diet with 30% crude protein before the commencement of the experiment. Fifteen juveniles fish (11.5±0.8 g) were randomly distributed into each happa net. There were two replicates per treatment and the fishes were fed twice daily at 07:00-08:00 and 16:00-17:00 h.

Diet formulation: The feed ingredients were purchased from FUNAAB Agro-Allied Company mill, Abeokuta while biscuit waste, Indomine® (noodles) and Gala® (sausage roll) waste were gotten from the Industrial Estate in Lagos. The compounded feed was pelletized indoor using a hand pelletizer with a 2 mm diameter and dried.

Experimental procedure: Fish in each treatment were fed with experimental diet at 3% of their body weight. Weight changes were recorded weekly with a sensitive electronic weighing scale (Meter Toledo FB602) and feed adjusted appropriately. At the end of 8 weeks the effect of these experimental diet were observed.

Physico-chemical analysis: The physico-chemical parameters of experimental set-up such as pH, conductivity, dissolved oxygen, Total Dissolved Solids (TDS) and Temperature were monitored weekly throughout the period of the experiment.

Haematological examination: At the end of the week 8 of feeding, fish from each treatment were randomly selected for haematological analysis. Blood was drawn from the caudal vein through the lateral line of fish into ethylenediamine tetra-acetic acid EDTA bottles by the use of needle and syringe for determination of the Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) and biochemistry:

Packed cell volume (PCV): The heparinized capillary tubes were 3/4 filled with whole blood and one end sealed with plasticine. The tubes were centrifuged for 5 min in a microhaematocrit centrifuge at 12,000 rpm. The PCV was read by the use of haematocrit reader (Kelly, 1979).

Red blood cell (RBC) and white blood cell (WBC) counts: The RBC and total WBC counts were carried out by use of the Neubauer improved counting chamber as described by Kelly (1979). For red blood cell counts, blood was diluted 1:200 with Dacies fluid (99 mL of 3% aqueous solution of sodium citrate; and 1 mL of 40% formaldehyde) which keeps and preserves the shape of the red blood cell for estimation in the counting chamber (Kelly, 1979).

Total white blood cell counts: For white blood cell counts, the dilution was 1:20 using 2-3% aqueous solution of acetic acid to which tinge of Gentian violet was added. Thin blood smears were stained with Wright-Giemsa stain (Schalm *et al*., 1975). A total of 100 white blood cells were enumerated and differentiated.

Haemoglobin (Hb) estimation: The cyanmethemoglobin method as described by Schalm *et al.* (1975) and Kelly (1979) was used in the determination of haemoglobin concentration. Well-mixed blood of 0.02 mL was added to 4 mL of modified Dabkin's solution (potassium ferricyanide, 200 mg; potassium cyanide, 50 mg; potassium dihydrogen phosphate 140 mg. The volume was made up to 1 L with distilled water at pH of 7.0. The mixture was allowed to stand for 3 min and the Hb concentration was read photometrically by comparing with a cyanmethemoglobin standard with a yellow-green filter at 625 nm.

Biochemical analysis

Total protein: The total protein was determined by the interaction of cupric ions in an alkaline solution with protein peptide bonds resulting in the formation of a coloured complex, the absorbance is read spectrophotometrically at 546 nm (Tietz, 1995):

$$\text{Total protein} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (g L}^{-1}\text{)}$$

Alkaline phosphatase: The Alkaline phosphatase was determined by an optimized standard method according to the recommendations of the deutsche gesellschaft fur klinische chemie:

$$\text{p-nitrophenylphosphate} + H_2O \rightarrow \text{phosphate} + \text{p-nitrophenol}$$

Determination of alanine aminotransferase (ALT): ALT was determined using commercial kit Randox® based on the principle described by Schirmeister (1964), Henry (1974) and Tietz (1995).

Determination of aspartate aminotransferase (AST): It was determined using commercial kit Randox® based on the principle described by Schirmeister (1964), Henry (1974) and Tietz (1995).

Albumin (ALB): The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3', 5, 5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the sample using commercial kit Randox®.

Growth performance parameters: The effect of various treatments on growth performance of fish was defined using mean weight gain, average daily growth, specific growth rate, feed conversion ratio, feed efficiency ratio and protein efficiency ratio.

Mean weight gain: Mean weight was computed as the average weight gained by the experimental fish in the course of the feeding trial:

$$\text{Mean weight gain (g)} = \text{Final mean weight (g)} - \text{Initial mean weight (g)}$$

Specific growth rate (SGR): Specific growth rate:

$$\text{SGR (\%/day)} = \frac{\log W_2 - \log W_1}{T_2 - T_1} \times 100$$

where, W_1 is the weight at time T_1 and W_2 is the weight at time at T_2.

Feed conversion ratio (FCR):

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total dry feed fed (g)}}{\text{Total wet weight gain (g)}}$$

Protein efficiency ratio (PER): This is the relationship between wet weight gain of fish and the protein content of feed:

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Amount of protein fed (g)}}$$

Statistical analysis: Data collected were subjected to analysis of variance (SAS Institute, 1999). Duncan's multiple range tests was used to compare means among treatments at a probability level of 5%.

RESULTS

The experimental diets were well accepted by the fish species. No mortality was observed as a result of tolerable physico-chemical water parameters throughout the experimental period. The haematology study observed no stress or pathological sign while the growth response revealed that the waste feeds could adequately replace maize fraction of the fish diet.

Table 1 shows the percentage gross composition of each experimental diet; Table 2 records the proximate composition of ingredients-maize and feed wastes. Maize has the highest values in moisture content (8.97), Ash (7.06) and carbohydrate (39.89). Indomie has the lowest moisture (7.66) and maize low fat content (2.24). Crude fibre is high in indomie (7.68) while crude protein is likewise high in Gala (39.21). Table 3 shows the Mean weekly values of physico-chemical parameters during the experimental period.

Table 4 recorded the growth response of the fish fed with the different experimental diets. The weight gain in the experimental feed fed with gala waste was significantly different ($p<0.05$)

Table 1: Gross composition of experimental diet

Components	TD1	TD2	TD3	TD4
Maize	23.37	-	-	-
Biscuit waste meal	-	23.37	-	-
Indomine	-	-	23.37	-
Gala	-	-	-	23.37
Soybean meal	27.56	27.56	27.56	27.56
Fish meal	27.56	27.56	27.56	27.56
Groundnut cake	14.26	14. 26	14. 26	14. 26
Vegetable oil	5.00	5.00	5.00	5.00
Calcium phosphate	0.50	0.50	0.50	0.50
Vitamin C	0.10	0.10	0.10	0.10
Salt	0.15	0.15	0.15	0.15
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Premix*	1.00	1.00	1.00	1.00

*Contains Vit. A 4000000 IU, Vit. D: 800000 IU, Vit. E: 40000 mg, Vit. K: 3800 mg, Vit. B1: 1000 mg, Vit. B2: 6000 mg, Vit. B6: 5000 m, Vit. B12: 25 mg, Niacin: 6000 mg, Patothenic acid: 20000 mg, Folic acid: 200 mg, Folic acid: 200 mg, Biotin: 8 mg, Manganese: 300000 mg, Iron: 80000 mg, Zinc: 20000 mg, Cobalt: 80 mg, Iodine: 400 mg, Selenium: 40 mg and Choline: 800000 mg

Table 2: Proximate composition of feed ingredients

	Moisture (%)	Dry matter (%)	Fat (%)	Ash (%)	Crude fibre (%)	Crude protein (%)	Carbohydrate (%)
Maize	8.97	91.030	2.24	7.06	6.14	36.72	39.89
Biscuit	8.74	91.260	5.65	6.89	5.83	35.94	38.13
Indomine	7.66	92.340	5.78	6.86	7.68	35.62	39.56
Gala	8.57	9.143	3.94	6.95	5.27	39.21	36.61

Table 3: Mean weekly values of physio-chemical parameters during the experimental period

Weeks	pH	Dissolved oxygen (mg L^{-1})	Temperature (°C)
0	7.10	8.0	27.10
1	6.90	7.7	26.00
2	6.96	8.5	26.75
3	7.54	7.3	28.00
4	7.42	8.1	27.00
5	7.53	7.8	26.40
6	7.61	8.1	27.40
7	7.41	6.4	27.00
8	7.31	6.2	27.00

Table 4: Summary of the growth response in *Clarias gariepinus* for 8 weeks experimental period

Parameters	Weight gain (g)	Feed intake (g)	Protein intake	PER	FCR	SGR (%/day)	Survival (%)
Maize (control)	119.70±4.09^{b}	126.84±13.02^{a}	50.74±5.20^{ab}	2.34±0.15^{a}	1.05±0.10^{a}	4.53±0.21^{a}	100
Biscuit	108.67±6.81^{b}	138.1±10.720^{ab}	55.24±4.29^{ab}	1.99±0.12^{a}	1.26±0.05^{a}	4.47±0.91^{a}	100
Indomie	107.67±6.05^{b}	114.4±12.390^{b}	45.76±4.96^{b}	2.37±0.16^{a}	1.06±0.08^{a}	4.30±0.19^{a}	100
Gala	138.71±4.34^{a}	153.89±12.26^{a}	61.56±2.50^{a}	2.28±0.17^{a}	1.11±0.04^{a}	4.63±0.15^{a}	100

Means with the same superscript in the same column are not significantly different ($p>0.05$), PER: Protein efficiency ratio, FCR: Feed conversion ratio and SGR: Specific growth rate

(138.71±4.34), followed by control diet Maize (119.7±4.09). Likewise the feed intake is significantly different in Gala waste ($p<0.05$) from other feeds having (153.89±12.26) followed by Biscuit

Table 5: Haematological parameters observed in *Clarias gariepinus* fed with diet for 8 weeks

Parameters	Control	Biscuit	Indomine	Gala
PCV	37.00±1.82[a]	34.40±3.00[a]	36.40±2.67[a]	34.00±1.38[a]
HB	10.22±0.29[a]	9.70±0.49[a]	9.40±0.46[a]	9.30±0.33[a]
RBC	2.26±0.17[a]	2.02±0.23[a]	2.18±0.13[a]	2.28±0.20[a]
WBC	11.76±0.47[a]	10.06±1.10[a]	10.80±0.64[a]	11.42±0.93[a]
PLAT	203.40±15.18[a]	189.60±27.09[a]	206.40±4.15[a]	207.20±16.76[a]
TP	3.04±0.18[a]	2.47±0.12[a]	3.40±0.32[a]	3.44±0.39[a]
ALBU	2.06±0.21[a]	1.60±0.20[a]	1.74±0.14[a]	2.12±0.37[a]
AST	45.80±6.85[a]	49.60±4.812[a]	42.60±9.212[a]	52.40±4.411[a]
ALT	13.80±2.31[a]	17.60±2.09[a]	15.20±2.56[a]	15.80±1.72[a]
ALP	43.20±3.94[a]	45.20±3.81[a]	50.00±4.58[a]	46.40±4.03[a]
GLOB	0.98±0.21[a]	1.14±0.16[a]	1.46±0.15[a]	1.32±0.23[a]
HCT	38.80±3.84[a]	38.40±2.18[a]	46.60±5.23[a]	45.80±4.20[a]
LYM	53.00±3.67[a]	55.20±3.38[a]	44.60±4.81[a]	47.40±5.00[a]
EOS	1.00±0.45[a]	0.60±0.25[a]	0.80±0.37[a]	0.60±0.60[a]
BAS	0	0	0	0
MON	7.20±1.86[a]	5.80±2.11[a]	6.00±1.34[a]	5.40±1.29[a]

Means with the same superscript in the same column are not significantly different (p>0.05)

Table 6: Cost analysis of the feed ingredients

Feed ingredients	Cost kg^{-1} (Naira)	Cost kg^{-1} (dollars)
Maize	80	0.53
Biscuit waste	35	0.23
Indomine waste	40	0.27
Gala waste	40	0.27

(138.1±10.72) and the least Indomie (114.4±12.39) in the experiment. There were significant difference ($p<0.05$) in the protein intake of the experimental feed while fish fed with gala waste had the highest protein intake (61.56±2.5), followed by Biscuit (61.56±2.5) and control maize (50.74±5.20), respectively. Protein efficiency ratio with biscuit waste was low (1.99±0.12), highest in Indomie (2.37±0.16) but not significantly different ($p>0.05$) in all the diets. The feed conversion ratios and the specific growth rates of the waste feeds were not significantly different ($p>0.05$) from the control feed. The experiment recorded 100% fish survival in all the diets. The results of the haematological analyses are presented in Table 5. Gala recorded highest value in platelet, total protein, albumin, aspartate aminotransferase and the control diet recorded highest in packed cell volume, haemoglobin, white blood cell but there was no significant difference ($p>0.05$) in all the haematological parameters observed. This study has provided valuable baseline data on the haematology of *Clarias gariepinus*

Cost Analysis of different feed ingredients is presented in Table 6. Biscuit is the cheapest (0.23 USD), followed by Gala and Indomie (0.27 USD) and the highest cost is the maize (0.53 USD).

DISCUSSION

The experimental fish species (*Clarias gariepinus)* grew well and survived with the experimental diets which signify that waste food ingredients are nutritious, adequate and healthy for feeding fish. This is in correlation with the report of Ayoola (2010) and Ajasin *et al.* (2010) where *Clarias gariepinus* was fed with poultry waste and biscuit waste replaced maize in the diet

of snail, respectively. In other word this study shows that biscuit waste, Indomine® and Gala® can replace maize in the diet of fish. This collaborate the report of Alegbeleye *et al.* (2008) where maize was replaced with boiled *Colocassia esculenta* flour. The fish fed with biscuit waste and Gala® had the feed intake which is significantly different compared to the other feeds; this might be as a result of their palatability which was reported by Eniolorunda (2011). The inclusion rate of Gala® waste into the feed to replace maize is a promising alternative having higher weight gain, feed intake and protein intake than the costly control feed ingredient (maize). Furthermore, the feed conversion ratio of Gala® (1.11±0.04) is substantial for a favourable replacement in terms of growth. This might be attributed to the high crude protein (39.21%) of Gala® in the proximate analysis (Table 2). The significant difference ($p<0.05$) observed in the weight gain and protein intake of the experiment confirmed that waste feed can be economical in terms of the cost of feed production.

The waste meal was analysed and found to contain substantial amount of nutrients such as protein, energy and minerals required for animal growth and performance (Longe, 1986; Olayeni *et al.*, 2007), this statement is being confirmed by this study since there is no significant different in the FCR and SGR throughout the 8 weeks experimental period.

The cost implication therefore reveals that the use of any of these wastes will reduce cost of feed production by at least 30% since maize accounts for up to 60% of feed.

The physico-chemical parameters of water were within the range for culturing African catfish, *Clarias gariepinus* (Viveen *et al.*, 1977). (Abo-Esa, 2008) recommended Dissolved Oxygen (DO) level of between 4-8 mg L^{-1} in ponds and DO values observed during the experimental period fall within these values.

Haematology is important in the diagnosis of fish diseases and assessment of the effect of feed waste. Haematological effect of different feed waste on the juvenile of *Clarias gariepinus* at 8 weeks with no significant difference of erythrocyte count (RBC) and haematocrit value in response to the present experiment may be attributed to the fact that these wastes have no antinutritional factors or toxicity. This is to confirm the report of Eniolorunda (2011) that biscuit waste meal has no antinutritional factor and could make a good replacement for maize and other cereal grains in fattening ram for market or for slaughter. Unlike what was observed in *C. gariepinus* after ochratoxin intoxication where there was decrease in the RBCs, haemoglobin and PCV (Ramadrvi *et al.*, 1998; Mousa and Khattab, 2003). Also WBC with its major function to fight infection, defend the body by phagocytosis against invasion by foreign agents and to produce or at least transport and distribute antibodies in immune response are not significantly different across the group.

Total Protein (TP) concentrations depend on the structural liver alterations that reduce aminotransferase activity, with concurrent reduction in deamination capacity (Burtis and Ashwood, 2001). Table 5 reveal that TP value was low in biscuit waste (2.47±0.12). Although, not significantly different, this may be as a result of high sugar and salt content in the biscuit waste. The same was observed by Odunsi *et al.* (2007) on the potential of maize, sorghum, millet and biscuit waste meal as energy sources in diets of laying Japanese quails. There was no significant difference ($p>0.05$) in the Albumin, globulin Aspartate amino transferase (AST) and Alanine amino-transferase (ALT) of the fish in the control diet compared with the other experimental diet, being the most abundant protein in serum.

From the count of neutrophils, lymphocytes, monocytes, eosinophils and basophils shows that there is no significant difference from the fish fed with control diet compared to others fed with the waste diet.

CONCLUSION AND RECOMMENDATION

In view of the growth performance, survival rate and haematological study of this research, it is concluded that waste industrial food ingredients could favourably replace maize in the diet of *Clarias gariepinus* fish species. The biscuit, indomine and gala wastes are readily available in respective industries and their inclusion into fish feed as substitutes for conventional feed ingredients reduces cost of feed with encouraging growth rate performance.

Moreover, this research feed formulation would enhance profit margins in fish culture in Nigeria and would not permit too much dependence on commercial feeds which brings a lasting solution to the high cost and shortage of commercial feeds for aquaculture ventures in Nigeria. Constraints on the successful operation of intensive aquaculture business as a result of high feed cost is minimised with the results of this research.

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