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Evaluatoin of Biochemical Changes Associated with Replacement of Maize with Whole Cassava Root Meal in the Diet of Hybrid Catfish

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Abstract: This study was done to evaluate the biochemical changes associated with the replacement of maize with varying levels of whole cassava root meal in the diet of hybrid catfish. Four diets with varying replacement levels of maize with whole cassava root meal at 0% (A_0), 33% (B_{33}), 66% (C_{66}) and 100% (D_{100}) were formulated and fed to hybrid catfish fingerlings for a period of 32 weeks. And the following biochemical properties were evaluated: serum metabolites namely creatinine, urea, total protein, bilurubin a. and b., while for serum enzymes aspartate aminotransferase (AST), alanine amino transferase (ALT), Alkaline phosphatase (ALP) and Lactate Dehydrogenase (LDH). The results indicated that creative and total protein were significant ($p>0.05$) in all the treated diets and had a concentration depended increase above the control values. Whereas the reverse was the case with bilirubin b., which was higher in the control than in the treated group. For the serum enzymes evaluated in this trial, replacement of maize with whole cassava root meal caused a significant declined ($p<0.05$) in serum aspartate aminotransferase (AST), alkaline phoslatase (ALP) and Lactate Dehydrogenase (LDH) at all levels. While the values of Alamine amino transferase (ALT) increased considerably ($p<0.05$). The mean values of these metabolites and enzymes were within the acceptable range for normal metabolism of hybrid catfish. It can therefore be concluded that whole cassava root meal can adequately replace maize without any adverse effects on the biochemical properties of the fish.

Key words: Biochemical, changes, enzymes, metabolites, hybrid, catfish, maize, cassava root meal

INTRODUCTION

The evaluation of feed ingredients is crucial to nutritional research and feed development for aquaculture species (Glencross *et al*., 2005). There are several important components that should be understood to enable judicious use of a particular ingredient in feed formulation. This includes information on digestibility, palatability and its effect on biochemical properties (Andrews *et al*., 1978; Rouhonen *et al*., 2001). Diet design, feeding strategy, faecal collection method all have important implications in the determination of the nutritional values of nutrients from any ingredient. The palatability of ingredients can be assessed in several ways. Usually, it is done by including various levels of the ingredient in question in a reference diet and the performance of the fish assessed. However, the design in replacement of ingredient can all be subject to some variations which depend on the species, level of replacement, growth performance and physiological status (Baber *et al*., 1988; Glencross *et al*., 2007).

Other issues such as ingredient functionality, influence on immune status and effects on metabolites, enzymes and blood tissue are very important considerations in determining the effectiveness of ingredients for replacement in formulation of aquaculture feed (Glencross *et al*., 2007). For any ingredient therefore to replace another effectively, it must satisfy the nutritional and physiological needs of the fish, up to 70% and above (Glencross *et al*., 2006).

Recent years marked a major shift in human understanding of the role of food productions in maintaining the health status of aquatic organisms (Stepanowska and Sawicka, 2006). Particular interest

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has been focused on haematological parameters (Erondu *et al*., 1993; Musa and Omoregie, 1999; Gabriel *et al*., 2007), enzyme profiles (Oruc and Uner, 2001) and metabolites (Begun, 2004).

The values of these biochemical variables may be influenced by species, water quality (Hrubec *et al*., 1997; Davis, 2004), age of fish (Hrubec and Smith, 2004) culture system (Hrubec, 2000), sampling method and diet (Sakamoto, 2001). Osuigwe *et al*. (2005) observed that the inclusion of different levels of raw and boiled jackbean, *Canavalia ensiforms* meal in the diet of hybrid catfish for 56 days induced an increase in the activity of alamine transaminase (ALT), aspartate transaminase (AST) and Glutamate Dehydrogenase (GDH), while Baber *et al*. (1988), recorded that channel catfish *Channa punctatus* fed pigeon pea, *cajanos* sp. altered the level of total protein ($p<0.05$), total free amino acids, nucleic acids, glycogen, pyruvate, lactate and activity of protease, alamine amino trasnferase, acetylcholinesterase and cytochrome oxidase enzyme in liver and muscle tissues of the fish. This study therefore, examines critically the biochemical changes associated with replacement of hybrid catfish a popular fish for culture in Nigeria which hitherto has not been reported.

MATERIALS AND METHODS

Nine hundred fingerlings of hybrid catfish were fed with four different diets with varying replacement levels of maize with whole cassava root meal.

These diets were formulated and designated A_o, B_{33}, C_{66} and D_{100} (Table 1). Diet A_o, which is the control had maize as the main source of energy. In diets B_{33}, C_{66}, D_{100} maize was substituted with whole cassava root meal at graded levels of 33, 66 and 100%, respectively. The experimental fish were reared in 12 different tanks of dimension (2.5×2×1.3 m). The tanks were labeled based on treatment levels and replicated with each diet having three tanks each.

Table 1: Percentage composition of experimental diets

Ingredients	A_o (control)	Diets B_{33}	C_{66}	D_{100}
Maize meal	13.18	8.49	4.11	-
Whole cassava root meal	-	4.25	8.21	11.94
Fish meal	27.75	27.90	27.00	27.00
Soya bean meal	41.64	41.00	42.07	42.26
Groundnut cake	13.88	14.81	15.06	15.25
Bone meal	2	2	2	2
Fish premix*	0.25	0.25	0.25	0.25
Methoionine	0.2	0.2	0.2	0.2
Lysine	0.3	0.3	0.3	0.3
Palm oil	0.3	0.3	0.3	0.3
Vitamin C	0.3	0.3	0.3	0.3
Common salt	0.2	0.2	0.2	0.2
Total (kg)	100	100	100	100
Cost (₦ kg^{-1})	183	182	178	176
Cost reduction (₦ ton^{-1})	0	1000	5000	7000
Energy (Kcal $kg\ Me^{-1}$)	3183	3097	3090	3079

Subscript in the diets indicate level of replacement of maize with whole cassava root meal; Fish premix, Each 2.5 kg contains, *Vitamin 8,000,000 IU, Vitamin D_3 1,600,000 I.U, Vitamin E 6,000 I.U, Vitamin K 2,000 mg, Thiamine B_1 1,5000 mg, Riboflavin B_2 4,000 mg, Pyriodoxine B_6 1,5000 mg, Niacin 15,000 mg, Vitamin B_{1210mg}, Pantothenic Acid 5,000 mg, Folic Acid 500 mg, Biotin 20 mg, Choline Chloride 200 g, Antioxidant 125 g, Manganese 80 g, Zinc 50 g, Iron 20 g, Copper 5 g, Iodine 1.2 g, Selenium 200 mg, Cobalt 200 mg

Fish were fed at the same feeding frequencies twice daily 68 and 17 h at 5% body weight. They were fed for a period of 32 weeks where they were raised to adult size.

The water in the experimental tanks was changed every week and the following parameters namely pH, Dissolved Oxygen (DO), temperature, nitrite, ammonia and total hardness were evaluated every week. Water temperature was determined by mercury in glass thermometer. The pH was determined in situ in each of the tanks with a pH meter (Hanna products Portugal). The dissolved oxygen were estimated using the Winkler's method as described by APHA (1985). Nitrite, Ammonia and Water hardness were determined by using fish farmers water quality test kit model FF-IA.

At the end of the experiment blood was taken from the fish with 21G needle by cardiac puncture, the blood was immediately transferred to heparinised bottle and refrigerated for laboratory analysis.

Enzymes namely Akaline Phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) Lactate Dehydrogerase (LDH) were analyzed using the methods as described by Bessey *et al*. (1986).

While plasma metabolites such as creatinine, total bilirubin, total protein and total area were analysed. In he laboratory using the methods of Smith 1981 and the values taken. The data obtained from the study were then analyzed accordingly to the statistical methods as described by Wahua.

RESULTS

The result obtained from the analysis of water quality variables indicted that pH ranged from 6.60-8.55, while dissolved oxygen ranged from 4.99-7.10 mg L^{-1}. Temperature ranged from 27.11-29.14°C, nitrite ranged from 0.0010-0.0054 mg L^{-1}, ammonia ranged from 0.30-0.46 mg L^{-1} and total hardness ranged from 46.05-80.06 (Table 2).

Table 2: Mean values of physiochemical parameters of water in the experimental tanks

		Range	
Parameters	Mean±SD	Minimum	Maximum
pH	6.56±0.4	6.60	8.58
Dissolved oxygen (mg L^{-1})	5.77±1.74	4.99	7.10
Temperature (°C)	28.14±0.11	27.11	29.14
Nitrite (mg L^{-1})	0.0039±0.01	0.0010	0.0054
Ammonia (mg L^{-1})	0.35±0.01	0.30	0.46
Total hardness (mg L^{-1})	50.11±10.12	46.05	80.08

Table 3: Serum metabolites of hybrid catfish fed experimental diets for 32 weeks (Mean±SD)

Parameters	Experimental diets			
	A_o	B_{33}	C_{66}	D_{100}
Creatinine (μmol L^{-1})	46.03±31.30^{bc}	36.32±10.53^{c}	72.17±29.21^{ab}	80.77±7.13^{a}
Urea (mmol L^{-1})	1.08±0.17^{b}	1.10±0.24^{a}	1.16±0.23^{ab}	1.23±0.15^{ab}
Protein (g L^{-1})	38.27±12.56^{a}	42.58±5.03^{a}	44.41±5.88^{a}	45.62±4.39^{a}
Bilurubin a. (μmol L^{-1})	5.78±1.93^{a}	9.08±6.58^{a}	6.18±4.38^{a}	5.05±3.44^{a}
Bilurubin b. (μmol L^{-1})	3.08±1.54^{a}	1.28±1.92^{a}	2.25±2.46^{a}	2.26±3.51^{a}

Mean with the same superscript within the row are not significantly different (p>0.05)

The mean values of creatinine obtained in the study, ranged from 36.32±10.53 μmol L^{-1} in diet B_{33} to 80.77±7.13 μmol L^{-1} in diet D_{100} (Table 3). However, there was significant difference (p<0.05) of mean values of creatinine in all the experimental diets (Table 3).

The mean values of urea obtained in the metabolites analysis of the experimental fish increased significantly (p<0.05) across the dietary treatments, with the highest value 1.23±0.15 mmol L^{-1} observed in diet D_{100}, while the lowest 1.08±0.17 was recorded in diet A_o (Table 3). The protein mean values recorded during the metabolites analysis of experimental fish ranged from 38.27±12.56 g L^{-1} to 45.62±4.39 g L^{-1}, with no significant difference between the dietary treatments (Table 3).

Diet B_{33} had the highest value 9.08 of Bilurubin a. (9.08±6.58 μmol L^{-1}), while the lowest value 5.05±3.44 μmol L^{-1} was observed in diet D_{100} (Table 3). The mean values ranged from 1.28±1.92 μmol L^{-1} in diet B_{33} to 3.08±1.54 μmol in diet A_0. However there was no significant difference (p>0.05) in all the experimental diets (Table 3).

The highest mean values of AST, 9.23±3.54 μ L^{-1} were recorded in diet A_o and the lowest 5.12±5.72 μ L^{-1} in diet B_{33}. The result recorded significant different (p<0.05) between the treated groups and control (Table 4). The mean values of ALT ranged from 1.96±2.73 μ L^{-1} observed in diet B_{33} to 3.48±2.80 μ L^{-1} recorded in diet A_o. Although, the result recorded significant difference (p<0.05) between the treated groups and control, there was no significant difference (p>0.05) between diet A_o and C_{66} (Table 4). The highest values of ALP 6.30±3.18 μ L^{-1} was recorded in diet A_o, while the lowest value 3.07±1.14 μ L^{-1} was observed in diet C_{66}. There was significant difference (p<0.05) between treated groups and control (Table 4).

The control diet A_o had the highest value 32.83±13.84 μ L^{-1}, while the lowest value 17.33±11.79 μ L^{-1} was observed in diet B_{33}. Treated groups were significantly different (p<0.05) from control (Table 4). The LDH increased with increase in WCRM inclusion.

Table 4: Serum enzymes of hybrid catfish fed experimental diets for 32 weeks (Mean±SD)

Parameters (μ L^{-1})	Experimental diets			
	A_o	B_{33}	C_{66}	D_{100}
AST	9.23±3.54^{a}	5.21±5.72^{b}	5.53±5.06^{b}	6.05± 3.85^{ab}
ALT	3.48±2.80^{a}	4.96±2.73^{b}	4.35±4.02^{a}	4.12±3.22^{b}
ALP	6.30±3.180^{a}	5.81±3.95^{b}	3.07±1.14^{d}	4.52±2.91^{c}
LDH	32.83±13.84^{a}	17.33±11.79^{c}	19.66±15.30^{c}	28.00±38.58^{b}

Mean with the same superscript within the row are not significantly different (p>0.05); AST: Asparate aminotransferase; ALT: Alanine aminotransferase (ALT); ALTP: Alkaline Phosphatase; LDH: Lactate Dehydrogenase

DISCUSSION

There are a number of reasons for attempting to correlate fish feed with measurable characteristics of the animals tissue (Xiajun and Ruyung, 1990). On one level, the recent increase in understanding of the rates of protein turnover underlying protein formation has been accompanied by a number of studies demonstrating that rates of protein synthesis are closely correlated with the synthetic capacity of the tissue (Houlihan *et al*., 1993). The water quality parameters obtained in this research is within the acceptable range for fish culture. There is a need to expand our understanding of the mechanisms controlling the growth rate of fish, as protein synthesis is a major contribution to energy metabolism in fish.

The serum metabolites (urea, protein, bilurubin a and b) are indicators of adequacy of protein in terms of quality and quantity in the diet. These variables indicate the protein state of the fish in terms of protein malnutrition, alterations in the dietary intake of protein and pattern of utilization and possibly the extent of muscle wastage and subsequent degradation of muscle phosphorus and creatinine (Eggum, 1987). Although, cyanide which is found mainly in cassava may affect the pattern of protein utilization in animals (Iyayi and Tewe, 1992), there was no evidence in this study to suggest that the WCRM affected serum metabolites. The value obtained fall within the range recommended by Weatherley and Gill (1987) and Xiaojun and Ruyung (1990) for normal growing fish. The lack of variation (p>0.05) in total serum protein, urea, creatinine, bilurubin a and bilirubin b between the control and treatments suggests that these metabolites were not affected by the dietary treatments.

CONCLUSION

Blood enzyme profiles provide important information about the internal environment of the organism (Masopust, 2000). Enzymes activities affect various chemical and biological reactions in the body of the fish. According to Gabriel and George (2005), transamination is one principal pathway for synthesis and deamination of amino acids enabling carbohydrate and protein metabolism during fluctuating energy demands of the organism under adaptive conditions. Dietary treatment of hybrid catfish with WCRM led to decreased AST, ALT, ALP and LDH as was reported in study by Tackett *et al.* (1988), who reported same in channel catfish. Declined activites of these enzymes indicated that inactive transamination and oxidative deamination has taken place, as a result of the diversion of the alpha amino acids in the TCA cycle as keto acid to support energy production (Prasada and Ramana, 1985).

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