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Original Article

A Study on growth performance and proximate composition of African catfish (*Clarias gariepinus*) fed diets containing varying levels of vitamin A fortified cassava leaf meal

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ABSTRACT

This study was conducted to evaluate the growth performance and fillet quality of *Clarias gariepinus* fed varying levels of vitamin A fortified cassava leaf meal (VA-F-CLM) as a partial replacement for soybean meal. Four isonitrogenous experimental diets were formulated replacing soybean with vitamin A fortified cassava leaf meal at 0%, 5%, 10% and 15% (T1, T2, T3 and T4, respectively). A total of 120 juvenile *Clarias gariepinus* averaging 15±0.56g were randomly distributed into twelve aquaria at ten fish per aquarium, with three replicate aquaria for each experimental diet. During the feeding trial, fish were fed to satiation twice daily. At the end of a sixty-day feeding trial, weight gain and feed efficiency of fish fed T1 were significantly higher than values for fish fed T2, T3 and T4 ($P<0.05$). There was a difference ($P<0.05$) in weight gain, feed efficiency, protein efficiency ratio and feed efficiency ratio of fish fed experimental diets. Weight gain and feed efficiency of fish fed T2 were significantly higher than those of fish fed T3 and T4. Protein efficiency ratio of fish fed T1 was significantly higher than values for fish fed T3 and T4. Based on growth performance, VA-F-CLM could replace up to 5% of soybean protein. Dry matter and ash, increased ($P<0.05$) with increasing levels of dietary VA-F-CLM supplementation. Moreover optimum protein content in fish fillet was recorded with T2.

Key Words: Cassava leaf meal, vitamin A, growth performance, proximate composition

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1. INTRODUCTION

Feed is one of the major inputs in aquaculture production and fish feed technology is one of the least developed sectors of aquaculture particularly in Africa and other developing countries of the World (Gabriel *et al*., 2007). High cost of fish feed was observed as one of the problems hampering aquaculture development (Gabriel *et al.,* 2007). Expensive feeds will minimize or even nullify profit margins, reduce the potentials for capacity development of fish farms and associated investments (Adikwu, 1992). In recent years, research interest has shifted towards alternative sources of protein for the production of fish feeds. Some of these sources are largely non-conventional sources which are sparsely consumed by man. They are also vegetal in origin and include *Ipomoea batatas*, (Antia *et al*., 2006; Adewolu, 2008), Baobab, (*Adansonia digitata*) seed meal (Anene *et al.*, 2012), *Moringa oleifera* or 'drumstick (Yuangsoi and Masumoto, 2012) and *Gomphraena celosiodes* leaf meal (Anene *et al.* 2014). The major considerations for selecting these vegetable protein sources for the production of fish feeds include palatability, digestibility, and acceptability by the cultured fish (Dorothy *et.al.* 2018). Cassava is staple root crop cultivated and consumed in Nigeria. Cassava leaf meal is also known to posses protein at levels of 16.7–39.9% dry weight (Awoyinka *et. al.* 1995). These attributes of cassava has encouraged its use as an ingredient in the production of animal feeds including fish (Nnaji *et al*., 2010; Anyanwu *et. al.* 2012; Udo and John, 2015 and Hassan *et. al.* 2017). Recently, National Root Crops Research Institute (NRCRI) in Nigeria and the International Institute for Tropical Agriculture (IITA) introduced three new bio-fortified varieties of cassava namely NR07/0220, IITA-TMS-IBA070593, and IITA-TMS-IBA070539 that contain high amounts of beta-carotene, the substance that can be converted to Vitamin A in human body (Pfeiffer and McClafferty, 2008; Frano *et. al.* 2013). Literature abounds on the use of cassava leaf meal in the diet of *C. gariepinus* (Olurin *et. al* 2006, Bohnenberger *et. al.* 2008, Anyanwu *et. al.* 2012). However, there is a lacuna on the use of cassava leaf meal as a protein source and more so with the use of Vitamin A enriched (bio-fortified) cassava leaf meal in the diet of juvenile *C. gariepinus*. This study therefore investigated the effects on growth and proximate composition of *C. gariepinus* fed diets with varying levels of vitamin A fortified cassava (*Manihot esculenta*) leaf meal as a partial replacement for soybean meal, a more expensive protein source and to make recommendation based our findings.

2. MATERIALS AND METHODS

2.1.Experimental Site

The experiment was conducted in the Animal Nutrition Laboratory of the Animal Science and Fisheries Laboratory of Faculty of Agriculture, Abia State University Uturu, Umuahia Campus, Nigeria. The area is located on latitude $05^{0}27$ North longitude $07^{0}52$ East with an altitude of 122m above sea level. Umudike has an ambient temperature range of 22^{0}-37^{0}C with annual rainfall of 2,177mm and relative humidity of above 50-90%.

2.2.Experimental System

A total of 12 aquaria each of 50 liters capacity were used for the feeding trial. The experimental tanks were cleaned, disinfected and allowed to dry for 24 hours after which they were filled with 40 liters of untreated borehole water. The tanks were screened with a net to prevent escape of the fish and entry of predators. Three aquaria were used for each experimental diet.

2.3. Processing of vitamin A fortified cassava leaf mea (VA-F-CLM)l.

Fresh leaves of vitamin A enriched cassava (NR07/0220) were harvested from the teaching and Research Farm of Abia State University Unuahia, Abia State, Nigeria. They were chopped to pieces with a knife to facilitate drying under the sun and spread on a clean mat in a well-ventilated room for seven (7) days until the leaves became crispy.

The dried leaves were blended into a smooth paste in a 3.8 L kitchen-type blender (Warning Products, New Hartford, CT) with a sieve size of 3.15mm. The blender was thoroughly cleaned and dried between samples. The leaf meal was used to formulate 5 isonitrogenous (30-34% crude protein) *C. gariepinus* diets replacing soya bean at 0, 5, 10, and 15% inclusion levels. All experimental diets were analysed for crude protein, crude fiber, moisture and ash according to methods described by (AOAC, 2010).

2.4.Sourcing and acclimatization of experimental fish

A total of 1000 *Clarias gariepinus* juvenile were collected from a fish farm at Umudike, Abia State. They were transported in a 50 liters plastic container in Animal Science and Fisheries Laboratory of Faculty of Agriculture, Abia State University, Umuahia Campus and acclimatized for fourteen (14) days. During acclimatization, the fish were fed ad-libitum on a commercial fish feed (Coppens feed).

2.5.Stocking of experimental fish/Experimental Design

The fish were starved for 24 hours prior to the commencement of the feeding trial. The fish averaging 15±0.56g were randomly distributed at the rate of 10 juvenile per tank; the feeding trial was carried out in 12 plastic tanks, each containing 40 liters of water. They were four (4) treatments. The fish were fed to satiation twice daily.

The experiment was spread out in a completely randomized design (CRD). The model of the experiment was:

$Y_{ij}=U+T_i+e_{ij}$

Where

Y_{ij} = j^{th} observation made on i^{th} treatment

U = overall mean

T_i = effect of i^{th} treatment

E_{ij} = random error

2.3. Composition of Experimental diet/Feeding:

Table (1) shows the composition of the formulated experimental diets. The diets T_1 T_2, T_3, and T_4 contained 0, 5, 10 and 15% of vitamin A enriched (bio-fortified) cassava leaf meal.

Table 1: Chemical composition and levels of inclusion of Vitamin A fortified cassava leaf meal in fish diets

Ingredients	T_1 (0%)	T_2 (5%)	T_3 (10%)	T_4 (15%)
Maize	30.00	30. 00	30.00	30.00
Cassava	0.00	5.00	10.00	15.00
Soybean	45.30	40.30	35.30	30.30
Wheat residual	12.00	12.00	12.00	12.00
Palm Kernel Cake	5.10	5.10	5.10	5.10
Fish meal	5.00	5.00	5.00	5.00
Bone meal	1.00	1.00	1.00	1.00
Vitamin premix	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25
Palm oil	1.00	1.00	1.00	1.00
Total	100%	100%	100%	100%
Proximate composition of experimental diet				
Dry matter	89.06	88.88	87.95	88.65
Moisture	10.93	11.77	12.04	11.34
Ash	11.42	10.74	12.03	10.92
Crude fiber	6.58	6.98	7.22	7.85
Fat	4.14	3.90	4.05	3.82
Crude protein	34.52	33.16	32.68	30.35

Each diet was formulated and compounded separately. The mixture was made into pellets using manual pelleting machine of 2mm size. After pelleting, the feed was sun-dried and stored in a sack bag for further use. The fish were stocked, 10 fish in each plastic tank and were fed for sixty (60) days on diets containing varying levels of vitamin A fortified cassava leaf meal. Experimental fish were fed to satiation twice daily (7:30 am and 18:00 pm). The experimental aquaria were inspected daily to remove food wastes, phaecal remains and dead fish. Mortality was recorded as percentage of number of fish per treatment.

2.4. Water management:

On daily basis, water was filled up to 70-80% of the capacity of the tanks. Untreated borehole water was used for the experiment. Water was temporarily stored in 50 liters plastic containers and was transferred into the experimental tanks every morning.

2.5. Weight measurement

Before stocking, 10 fish were randomly selected from each treatment and weighed to the nearest milligram using a digital weighing balance (Super Camry) and mean calculated to obtain the initial mean weight (W_1).

At the end of feeding trial, ten fish randomly selected were weighed to obtain the final body weight (W_2).

These parameters were calculated according to the following equations:

Weight gain Wg, g = (W_2) –(W_1).

Specific growth rate SGR, % /day = [(ln W2-ln W1)/t] × 100. Whereas

ln: is the natural log. and t: is the time in days.

Feed efficiency ratio FER = Wg/ feed intake.

Protein efficiency productive PER= Wg/ Protein intake.

Mortality, % = (No. of dead fish/ No of fish stocked) × 100.

2.6.Chemical Evaluation of experimental diets and fish fillets:

Fresh blends made of fish fillets and experimental diets were analyzed for proximate composition. Moisture, crude protein, crude lipid and ash contents were determined according to AOAC (2010).

2.7.Statistical Analysis

The mean, standard deviation and analysis of variance (ANOVA) was computed using statistical package for social science (SPSS) version 21. Mean was separated using Duncan Multiple Range Test and the level of significance was set at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1.Growth Performance:

The growth performance of *C. gariepinus* fed various experimental diets was evaluated in terms of body weight gain (g), specific growth rate, feed efficiency and protein efficiency ratio and these data are presented in Table 2.

Mortality was between 4-10% and was highest amongst fish fed diet T_1 (control). There were significant differences among the treatments mean ($P<0.05$). The mean weight of *Clarias gariepinus* fish fed diet T_4 (236.66g) was low compared to the mean weight of experimental fish fed diets T_1, T_2 and T_3 (566.67g, 283.33g, 280.0g respectively). The weight gain of fish fed diet T_1 higher than fish fed other diets. The weight gain of fish fed diets T_2 and T_3 were not significantly different ($P<0.05$) but was higher ($P<0.05$) than those fed on Diet T_4. These indicate a decrease in weight gain with increase in inclusion of cassava leaf meal. This observation tallies with Anyanwu *et. al*. (2012). Cassava characteristically contains anti-nutritional properties that may hinder uptake of nutrient for growth and this may account for the reduced growth at higher levels of inclusion of vitamin A enriched cassava leaf meal.

Table 2: Growth performance of *Clarias gariepinus* fed graded levels of vitamin A fortified cassava leaf meal

Items	T_1 (0%)	T_2 (5%)	T_3 (10%)	T_4 (15%)	SEM
Mortality	**9**	**7**	**10**	**4**	**-**
$W_{1\ (g)}$	**150.00**	**150.00**	**150.00**	**150.00**	**0.00**
$W_{2\ (g)}$	**716.67^a**	**433.33^b**	**430.00^b**	**386.67^b**	**4.03**
W_g (g)	**566.67^a**	**283.33^b**	**280.00^b**	**236.66^d**	**3.36**
FER (%)	**93.30^a**	**75.91^b**	**62.37^c**	**45.09^d**	**1.59**
PER (%)	**3.41^a**	**2.55^{ab}**	**2.23^b**	**2.46^b**	**0.57**
SGR (%)	**5.29^a**	**2.26^b**	**1.74^c**	**1.58^d**	**0.87**

Values are means from triplicate groups of fish where the means in each row with different superscript letters are significantly different ($P<0.05$).

3.2.Feed efficiency:

There are differences ($P<0.05$) in feed efficiency ratio of experimental fish. The relatively low feed efficiency recorded in diet T_4 may be attributed to the presence of anti-nutrients in cassava leaf meal such as linamarin (Presston, 2004; Azaza *et al.*, 2015; Garcia *et al.*, 2015).

3.3.Protein efficiency ratio:

The results for protein efficiency ratio of fish fed experimental diet were 3.41% for T_1, 2.55% for T_2, 2.23% for T_3 and 2.46% for T_4. There were differences ($p<0.05$) among the treatment means. Fish in group T_1 and T_2 had the highest protein efficiency ratio. This showed that they had higher growth rate more than others fed diets T_3 and T_4. This probably accounts for the relative higher weight gain.

3.4.Specific growth rate:

The specific growth rates were 5.29, 2.26, 1.74 and 1.58 for T_1, T_2, T_3 and T_4 respectively. There were significant differences ($P<0.05$) in the specific growth rate in juvenile fish fed experimental diets with varying levels of cassava leaf meal. Fish fed diet containing 15% level of vitamin A fortified cassava leaf meal had the lowest mean of specific growth rate which may be as a result of the presence of hydrogen cyanide (HCN) in vitamin A fortified cassava leaf meal.

Feed stuffs which have anti-nutritional factors recorded poor growth performance in fish when supplemented at high levels (Ugwu et al., 2006; Adewolu, 2008). Fish in T_2 group had the highest mean specific growth rate from other treatment means. Richter *et al*. (2002) reported several index (from 1.76-5.04g kg $^{-1}$ day^{-1}) in order to asses a more convenient maintenance diet formulations in red tilapia.

3.5.Proximate composition of fillets of experimental fish:

The proximate composition of fillets of fish fed experimental diets is presented in Table 3. There were significant differences ($p<0.05$) among the mean values of moisture, ash, crude protein and fat in all treatments. Fish in T_1 (0% inclusion) group had the lowest moisture content while T_4 (15% inclusion) had the highest moisture content. The moisture content was 85.50% in T_2 and this value was in consonance with that reported for cat fish (Gallagher *et. al*. (1991). The protein content in T_2 was 58.26% and was the highest (P<0.05) compared with the other groups.

Table 3: Proximate composition of fillets of experimental fish

Items	T_1 (0%)	T_2 (5%)	T_3 (10%)	T_4 (15%)	SEM
Moisture	84.33^d	85.50^c	86.45^b	87.38^a	1.78
Ash	9.43^d	11.10^c	12.48^b	13.38^a	0.49
Crude protein	54.84^b	58.26^a	56.50^c	55.64^d	1.33
Fat	14.86^a	14.66^b	14.20^c	13.96^d	1.24

Values are means from triplicate groups of fish where the means in each row with different superscript letters are significantly different (P<0.05).

Results show that CP content was highest (58.26%) at 5% and lowest (55.64%) at 15% inclusion of VA-F-CLM. Ash content was lowest (9.43% in the control group 0% cassava meal and highest was noted in T_4 (15% cassava leaf meal. General, fish fed a diet containing 5% VA-F-CLM showed significantly different crude protein, content at different rates of inclusion of Vitamin A fortified cassava leaf meal.

4. CONCLUSION AND RECOMMENDATION

This feeding trial incorporated varying levels of vitamin A fortified cassava leaf meal in the experimental diets of juvenile *C. gariepinus*. The results obtained showed that the diets so formulated and evaluated were acceptable to the fish. Proximate composition of experimental diets conforms to the recommendation of optimum dietary requirements of juvenile *C. gariepinus* especially with respect to crude protein and moisture content. Results also show that incorporation of cassava leaf meal at 5% was most efficient than other levels especially with respect to growth parameters that (weight gain, specific growth rate) that were assessed and percentage of crude protein in fillets of experimental fish.

Based on the results obtained in this study, it was shown that vitamin A fortified cassava leaf meal can be used to replace soybean meal in feeding ration of juvenile *Clarias gariepinus* fish at 5% level of inclusion without any visible detrimental effect on the test organisms.

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