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Culture and Utilization of Tadpole as Animal Protein Supplement in the Diet of *Heterobranchus longifilis* Fingerlings

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Abstract: Two hundred and forty *Bufo maculata* tadpoles of weight range 0.038-0.045 g (mean weight 0.04±0.008) g and lengths 1.2-1.6 cm (mean 1.4±0.018 cm) were randomly selected from a breeding tanks (2.0× 2.0×1.0 m) and raised in outdoor concrete culture tanks (1.0×1.0×0.75 m) for 84 days and monitored for growth, productivity and nutrient utilization. Tadpole were harvested, processed into meal and used in compounding five experimental diets of 42.5% crude protein to replace fish meal at different inclusion levels of 0% (control), 25, 50, 75 and 100% fed to *Heterobranchus longifilis* fingerlings. The mean weight gain for cultured tadpole was 3.16 g/tadpole; specific growth rate 2.21% and feed conversion ratio, 1.04. The result of the feeding experiment shows that WG, RGW, SGR, FCR and feed intake favoured fingerlings fed 50 and 25% whole tadpole meal inclusion diets with insignificant difference $p<0.05$ compared to other treatments. Highest insignificant difference $p<0.05$ apparent crude protein digestibility of 90.88% was in 25% whole tadpole meal. Better cost benefit ratio was reported in 25 and 50% whole tadpole meal diets. Based on the result of this study, the replacement of tadpole meal is recommended to 50% inclusion levels in the diet of *H. longifilis* for better growth performance, feed utilization, health status and cost benefits.

Key words: Growth, utilization, haematology, cost benefit, *H. longifilis*

INTRODUCTION

The cost of feeding is a major factor affecting the development of aquaculture enterprise in Nigeria (Olomola, 1990). On this account, Falaye (1992) and Fagbenro *et al.* (2003) reported that the use of commercial pellets and supplementary fish feed accounts for about 60 and 40%, respectively of the recurrent cost of fish farming venture in Nigeria. In 1989, the presidential task force on alternative formulation of feed identified feeding of fish as one of the major problems facing aquaculture industry development in Nigeria and proffered possible solution geared towards increasing fish production from this sector. One of the suggestions made by this task force was on the utilization of non-conventional proteins supplements of both animals and plants origin in practical fish diet and the following alternative feed ingredients: grasshopper, garden snail, shrimps head, feather, termite, maggot, crab meal, defatted cocoa cake, yam peel, mussels, periwinkle, lizard, palm grub, earthworm and frog amongst others (Guerrero, 1981; Stafford and Tacon, 1985; Fagbenro, 1988; Idonibuoye-obu *et al.*, 1993; Ajah, 1998; Alegbeleye and Oresegun,1998; Akegbejo-Samson, 1999; Ugwumba *et al.*, 2001; Sogbesan *et al.*, 2005; Sogbesan and Ugwumba, 2006) had been studied as both protein and energy sources in fish feed.

Bufo maculata is the commonest species from the family Bufonidae found in the semi arid zone of Nigeria (Segun, 1989). This animal with very high reproductive rate does breed during the rainy season alone in the wild and hardly is there any competition for this resource by man in this part of the world. For proper utilization of this natural resource and limitation of its annual wastage, it could

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be diverted as fishmeal supplement in fish feed. The possibility of replacing fish meal with toad meal has been reported by Annune (1990). There is dearth of information on the culture, nutritive value and utilization of *B. maculata* as fish feed ingredients in literature hence there is a need for this study. The utilization of the tadpole stage will also go along way to control the population of this animal.

MATERIALS AND METHODS

Experimental

Culture of Tadpoles

Two males with two females were of Toad *(Bufo maculata)* of weight range 100-150 g were captured using hand nets at the bank of Kigera Reservoir of National Institute for Freshwater Fisheries Research (NIFFR) New-Bussa, Nigeria Hatchery Complex during their pairing activity at night using hand nets in June, 2005. They were allowed to mate in two outdoor concrete tanks of dimension 1.0× 1.0×0.75 m which bottom had been laid with sandy-loamy soil and the top screened with wire gauze. Seventy-two hours after hatching of the eggs, six hundred tadpoles of weight range 0.03-0.05 g (mean = 0.04±0.008 g) and length range, 1.2-1.6 cm (mean = 1.4±0.018 cm) were collected from the breeding tanks using scoop net and stocked into the outdoor concrete culture tanks, 1.0×1.0×0.75 m at the Hatchery complex of NIFFR at the rate of one hundred and twenty tadpoles of known weights and lengths per tanks. The tadpoles were fed first with plankton then with 40% crude protein diet for twelve weeks in concrete outdoor tanks. Total cropping of the tadpoles was done with Scooping net. Weekly sampling of weight gain was done for 12 weeks.

Processing of the Animal Feedstuffs and Feed Formulation

Harvested tadpoles were processed into dry meal and grounded to powered form, analyzed for proximate composition and used as substitute for fish meal at inclusion levels of 0, 25, 50, 75 and 100% to prepare five isonitrogenous (crude protein 42.5% and Isocaloric (gross energy-1900 kcal kg^{-1}) diets (TP$_1$ (control)-TP$_5$). These were compounded and fed 5% body weight to fingerlings of *Heterobranchus longifilis* at the stocking density of 20 fingerlings per 50 L plastic tanks in indoor mini-flow though system of the hatchery unit, of NIFFR for 10 weeks. Each experimental treatment was in triplicate. Each treatment was also monitored for mortality, dead fish were removed, counted, recorded and use in determining the survival rate (Table 1).

Table 1: Percentage composition of ingredients (g/100 g of ingredients) and production cost of whole tadpole meal-based diets for the feeding trial

Ingredients	TP$_1$ (Control)	TP$_2$	TP$_3$	TP$_4$	TP$_5$
Fish meal	30.00	22.50	15.00	7.50	0.00
Whole tadpole meal	0.00	7.50	15.00	22.50	30.00
Yellow maize	28.70	21.10	15.20	8.90	1.30
Groundnut cake	11.70	17.30	20.90	24.60	25.00
Soy bean meal	12.60	14.60	16.90	19.50	25.20
Blood meal	10.00	10.00	10.00	10.00	10.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
*Vitamin/Minerals premix	2.00	2.00	2.00	2.00	2.00
Palm oil	2.00	2.00	2.00	2.00	2.00
Common salt	0.50	0.50	0.50	0.50	0.50
Bone	1.00	1.00	1.00	1.00	1.00
Cassava starch binder	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated crude protein (%)	42.50	42.50	42.50	42.50	42.50
Calculated gross energy (kJ/100 g)	1900.00	1900.00	1900.00	1900.00	1900.00
P:GE (mg of protein kJ^{-1} of GE)	22.40	22.40	22.40	22.40	22.40
Production cost (N kg^{-1} of feed)	82.29	76.36	72.00	67.78	63.69
Inclusion of whole tadpole meal (%)	0.00	25.00	50.00	75.00	100.00

TP: Whole tadpole meal. *Vitamin and Minerals: Vitamin A-10,000,000 IU.; D3-2,000,000 IU.; E-23,000 mg; K3-2,000 mg; B1-3000 mg; B2-6000 mg; Nacin-50,000 mg; Calcium Pathonate-10,000 mg; B6-5000 mg; B12-25.0 mg; Folic acid 1,000 mg; Biotin-50.0 mg; Choline chloride-400,000 mg; Manganese-120,000 mg; Iron-100,000 mg; Copper-8,500 mg; Iodine-1500 mg; Cobalt-300 mg; Selenium-120 mg; Anti-oxidant 120,000 mg

Biochemical and Statistical Analysis

The experimental diets, experimental fish and fecal samples were analysed for proximate composition, gross energy according to Association of Analytical Chemist Methods (AOAC, 2000). Experimental fish were randomly sampled for haematological analysis. The weekly weights recorded and feed supplied were used to compute the growth, nutrient utilization and economic evaluation indices following method of Morais *et al.* (2001) and New (1989).

All data collected were subjected to analysis of variance (ANOVA). Comparisons among treatment means were carried out by one way analysis of variance followed by Tukey's test (0.05). Least Significance Differences (LSD) was used to determine the level of significance among treatments. Correlation and regression analysis was carried out to determine the relationship between the treatments and some of the parameters using SPSS 10.0 Windows 2000 and Graph pad Instat packages.

RESULTS AND DISCUSSION

The age of *B. maculata* shown in Fig. 1 increased from the left to right with their different external developmental features. High correlation ($R = 0.967$, $p<0.05$) existed between the logarithm of the weekly weight of the tadpole and the period of the experiment (Fig. 2). The experimental diets ether extract increased with increase whole tadpole inclusions and these are significantly different ($p<0.05$) (Table 2). A steady linear increase in the weekly weight of *H. longifilis* fingerlings fed the control and

Fig. 1: Different developmental stages in the cultured *Bufo maculata*

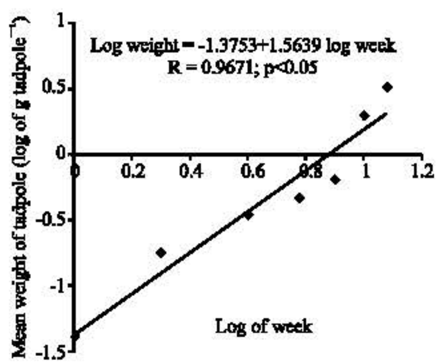

Fig. 2: Linear regression of bi-monthly weight of the *Bufo maculata* tadpole in an outdoor concrete tanks

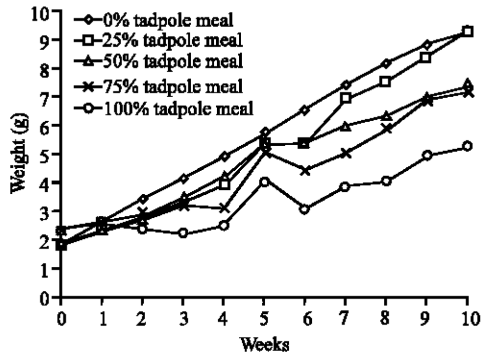

Fig. 3: Growth pattern of *Heterobranchus longifilis* fed whole tadpole meal based diets for 70 days

Table 2: Proximate and energy composition of whole tadpole meal and the experimental diets

	Tadpole meal					
Composition	Whole	0% (control)	25%	50%	75%	100%
Crude protein (%)	43.50	43.53	43.51	43.48	43.16	42.89
Ether extract (%)	11.30^{a}	10.63^{a}	11.62^{a}	13.10^{ab}	14.66^{bc}	16.07^{c}
Crude fibre (%)	34.75^{a}	3.36^{b}	3.58^{b}	3.74^{b}	3.92^{b}	3.99^{b}
Ash (%)	4.30^{a}	8.42^{b}	9.97^{c}	11.44^{d}	12.92^{e}	14.27^{f}
Nitrogen free extract (%)	3.35^{f}	18.33^{a}	15.92^{b}	13.11^{c}	10.51^{d}	6.87^{e}
Dry matter (%)	97.20^{a}	84.28^{b}	84.61^{b}	84.87^{b}	85.16^{b}	84.09^{b}
Gross energy kcal/100 g	1504.30^{a}	1732.04^{b}	1722.09^{b}	1732.90^{b}	1743.47^{b}	1732.45^{b}

All values on the same row with the different superscripts are significantly difference p<0.05

Table 3: Growth Indices of *Heterobranchus longifilis* fingerlings fed whole tadpole meal diets for 84 days

Indices	TP_1 control	TP_2	TP_3	TP_4	TP_5
Inclusion levels (%)	0	25	50	75	100
Mean initial weight (g fish^{-1})	1.87±0.06	1.83±0.39	1.94±0.06	2.35±0.2	2.30±0.04
Initial total length (cm fish^{-1})	6.2^{a}	6.6^{a}	6.7^{a}	7.6^{b}	7.2^{ab}
Mean final weight (g fish^{-1})	9.30^{a}	9.24^{a}	7.46^{b}	7.14^{b}	5.25^{c}
Mean weight gain (g fish^{-1})	7.43^{a}	7.41^{a}	5.52^{b}	4.79^{c}	2.95^{d}
Relative growth rate (% fish^{-1})	397.3^{a}	404.9^{a}	284.5^{b}	203.8^{c}	128.3^{d}
Specific growth rate (%)	0.995^{a}	1.00^{a}	0.835^{a}	0.689^{b}	0.512^{b}
Condition factor	1.30^{a}	1.22^{a}	1.35^{a}	1.05^{b}	0.92^{b}
Survival (%)	93.3^{a}	86.7^{b}	86.7^{b}	80^{c}	73.3^{d}
Feed conversion rate	3.23^{ab}	2.90^{a}	3.05^{a}	4.70^{b}	6.04^{b}
Protein retention (%)	50.33^{a}	44.55^{b}	47.89^{ab}	30.65^{c}	23.91^{d}
Protein efficiency rate	0.7923^{a}	0.7119^{b}	0.7832^{a}	0.4930^{c}	0.3862^{c}
Apparent net protein utilization (%)	34.40^{a}	32.23^{a}	33.49^{a}	25.08^{b}	20.73^{b}
Apparent digestible crude protein %	88.22^{a}	90.88^{a}	81.63^{b}	73.08^{c}	64.08^{d}
Haematocrit %	28.0^{b}	33.0^{a}	35.0^{a}	27.0^{b}	22^{c}
Haemoglobin (g dL^{-1})	10.34^{ac}	11.67^{ab}	12.36^{b}	10.09^{c}	9.36^{c}
White blood cell (×10^{3} dL^{-1})	2.80^{a}	2.90^{ab}	2.60^{a}	3.2^{b}	3.70^{c}
Profit index	1.54^{c}	1.70^{a}	1.64^{a}	1.54^{c}	1.61^{ab}
Incidence of cost	1.94^{a}	1.76^{c}	1.83^{b}	1.94^{a}	1.87^{b}
Benefit cost ratio	1.06^{a}	1.09^{a}	1.08^{a}	0.90^{ab}	0.80^{b}

All data with different superscript are significantly difference (p<0.05, p<0.001)

25% whole tadpole meal diets (Fig. 3). Highest specific growth rate, 1.00% day^{-1}, relative growth rate, 404.9% and best feed conversion ratio, 2.90 were from 25% whole tadpole meal diet (Table 3). The same diet also had highest apparent protein digestibility, 90.80%, best profit index, 1.70 and benefit cost ratio, 1.09. Fish fed 50% whole tadpole meal diet had best haematocrit, 35%, haemoglobin, 12.36 g dL^{-1} and white blood cell, 2.60×10^{3} dL^{-1} while the worst values, 22%, 9.36 g dL^{-1} and 3.70×10^{3} dL^{-1}, respectively which were significantly different, p<0.05 were from 100% whole tadpole meal diet.

The possibility of tadpole culture in isolation carried out in this study had earlier been reported by Benitez-Mandryano and Flores-Nava (1997) for the tadpole of *Rana catesbeiana* (Shaw). The fact that weight gain was reported from this experiment and low mortality is an indication that the tadpole like fish can also be raise in isolation and using concrete tank as against their natural earthen aquatic environment. Similar report had been made by Lutz and Avery (1999) on the possibility of culturing toad and frog in isolation. The mean weight gain reported in this study is lower to the mean weight gain of 3.65 g/ tadpole for reported by Benitez-Mandryano and Flores-Nava (1997). The different weight could be attributed to variation in growth of individual species which has also been reported in some other aquatic organisms like fish (Adeyemo *et al*., 1994; Nlewadim and Madu, 2004; Ojikutu *et al*., 2004) and not to the feed fed alone. The tadpoles were able to feed on compounded feed and used it for growth. Similar reported was documented by Benitez-Mandujano and Flores-Nava (1997) indicating its culture possibility even out of season.

The fact that weekly weight increase was recorded in all the treatments showed that the fish fed were able to utilized the feed for growth although the slopes showed a drop in week 2 for 100% whole tadpole meal diet. This may indicate that at higher inclusion level certain anti-growth factors were active in the feed (Obun *et al*., 2005) that the fish could not physiologically adjust. In the present study, the inclusion levels of whole tadpole meal appeared to be an important factor in influencing growth, acceptability index, feed conversion ratio, protein efficiency value, digestibility, energy retention, haematology, histopathology and the economics evaluation of the feeding *H. longifilis* than each index determined. This observation was in agreement with the report Annune (1990) that growth performance of *C. lazera* increased with increase in dietary inclusion of toad meal. The variation in the weight gain at different inclusion level of whole tadpole meal indicated that the meal contained certain toxins, which would have been introduced from the skin of the tadpole (Cochran, 1961).

The dietary energy content in the 42.5% crude protein diets appears to be optimum for maximizing feed efficiency and minimizing the body fat deposition in this study since Henken *et al*. (1986) had reported that when the protein-to-energy ratio is lower than the optimum value, fish weight is less at a given protein level. On the other hand an excessively high protein-to-energy ratio increases fatness in fish and reduces dress-out percentage. The high feed conversion ratio from this study indicate that lower quantity of the feed consumed was converted and utilized by the fish which could have been as a result of bitter taste that accompanied the presence of alkaloids and catecholamines (Harborne, 1991) reported in B. maculata (Sogbesan, 2007 Unpublished). DeSilva and Anderson (1995) reported food conversion values of *Clarias* as ranging from 3.2-6.7 according to the quality of the food given. In this study also, food conversion values ranged from 2.9-6.04 as the whole tadpole meal in the diets increased. The feed conversion ratio reported from this study is better than what was reported by DeSilva and Anderson (1995) since Adikwu (2003) had documented that the lower the feed conversion the feed then feed utilization. The result of protein efficiency rate and apparent net protein utilization showed an improvement in these indices as whole tadpole meal inclusion reduces. The fact that Apparent Net Protein Utilization (ANPU) was highly significant ($R = 0.997$; $p<0.01$) with protein efficiency rate appraised the protein quality of each of experimental diets. This ANPU is a factor of the digestibility, utilization and quality of the protein fed to the fish. Inclusion of whole tadpole meal at the lowest inclusion level showed a better ANPU may indicate that whole tadpole contain some proteolitic factors which limits its utilization at higher inclusion levels (Obun *et al*., 2005).

The haematological and blood chemistry result showed a negative effect at higher inclusion of this meal. The increase in whole-clotting time, white blood cell and blood glucose was an indication the apart from the feed delaying clotting ability of the fish blood, shooting up the immunity needs of the fish it also has adverse effect on the fish physiology (Spocrke, 2003). This report may be an improvement over that of Annune (1990) and Ayinla *et al*. (1994) that the fish fed were not diseased. Also the higher levels fed increase mortality which is an indication of the effect of the poison (Daly, 1995).

The substitution of whole tadpole meal for fishmeal lowers the cost of diet production which is an indication of a more cost efficient and cheaper non-conventional ingredient in relative to the fish meal. Economically, better cost benefit ratio reported in 25 and 50% whole tadpole meal diets and control indicated that at partial substitute levels of fish meal with whole tadpole meal, fish was able to maximize the utilization of the feed for muscle development at low cost (New, 1989; Mazid *et al*., 1997). Consequently, the farmer will benefit economically through the utilization of this cheaper ingredient (at 25% inclusion) to raise *H. longifilis* since there was no significant difference ($p>0.05$) between the weight gain at both control diet and 50% whole tadpole meal-based diet though better result could be obtained if the toxins in the skin which was believed to have cause reduction in the feed utilization and conversion are eliminated from this meal.

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