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Evaluation of Earthworm (*Hyperiodrilus euryaulos*, Clausen, 1914; Oligocheata: Eudrilidae) Meal as Protein Feedstuff in Diets for *Heterobranchus longifilis* Valenciennes, 1840 (Teleostei, Clariidae) Fingerlings Under Laboratory Condition

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Abstract: Isoproteic and isoenergetic diets containing 0% (control), 25, 50, 75 and 100% dried earthworm meal were fed to 250 fingerlings of *Heterobranchus longifilis* of weight range 2.35-3.03 g and average weight 2.68±0.29 g for 56 days experimental period. The results of the experiment showed that the water quality parameters were not affected as the inclusion level of the earthworm meal increased. The growth rates were not similar. The highest mean weight gain of 6.45 g $fish^{-1}$, relative growth rate of 240.67%/fish and specific growth rate of 0.9507%/fish were recorded in fish fed 25% earthworm inclusion diet. The lowest feed conversion rate of 1.51, highest protein efficiency ratio and productive protein value of 1.52 and 52.48, respectively were also recorded in fish fed 25% earthworm meal inclusion diet. The highest energy retention of 1.04 and lowest of 0.50 were recorded from 100% earthworm meal inclusion diet and the control, respectively. The lowest feed production cost of ₦56.32 k kg^{-1} of feed, lowest incidence of cost, 1.71 and highest profit index, 9.33 were recorded from fish fed 100% earthworm meal inclusion diet. Based on the result from this experiment 25% replacement of fish meal by earthworm meal is recommended in the diet of *H. longifilis* fingerlings for profitable and sustainable aquaculture practices.

Key words: Earthworm meal, growth, nutrient utilization, cost benefit *Heterobranchus longifilis*

INTRODUCTION

The increase in standard of living of man had led to increase in the prices of most of his consumable feedstuffs which are also used as conventional ingredients used in compounding fish feed. This cost factor has been estimated to account for about 60% of the total production cost in a functioning fish farm (Olomola, 1990). To curtail this and sustain intensive aquaculture practices, efforts are being enacted by various nutritionists to research and develop into the utilization of some non-conventional feedstuffs of both plant and animal origin such as grasshoppers, snails, shrimp head, feather, termites, tadpole, maggot, defatted cocoa cake, yam peel, cassava peel, palm grub and frog (Stafford and Tacon, 1985; Fagbenro, 1988; Ajah, 1998; Ugwumba *et al*., 2001; Madu *et al*., 2003; Omoyinmi, 2004; Sogbesan *et al*., 2005) to serve as both protein and energy sources in fish feed.

Heterobranchus longifilis is a highly priced tropical cultured fish with high growth rate, ability to thrive well in bodies of water not suitable for carp and tilapia, omnivorous feeding habit and desirable taste (Olufeagba, 1999). To meet the demand by consumers a cost-effective suitable diet with

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no anti nutritional factors (Sogbesan *et al*., 2006) but that is able to provide the required amino acids, fatty acids, dietary energy and vitamins is essential. Earthworm, which availability from natural habitat is seasonal but with prolific reproductive capacity (Razon-Arceno *et al*., 1981) has been reported to have amino acid composition similar to that of fish meal and potentially superior to meat meal (Dynes, 2003). Sogbesan *et al*. (2007) documented on the all year round production of earthworm, *Hyperiodrilus euryaulos* through vermiculture. The fact that the evaluation of this earthworm, *H. euryaulos* as fish meal supplement in the diet of *H. longifilis* has not been documented formed the basis for this study.

MATERIALS AND METHODS

Experimental System

A total of 15 indoor mini-flows through system with 50 L capacity, 0.25 m depth and 0.55 m diameter circular plastic tanks each were used for the trials. Water was supplied to each tank from 250 L head tank. The plastic tanks were cleaned, disinfected and allowed to dry for 24 h, after which water was supplied to two-third of the size of the tanks through the head tank and were covered with a net of mesh size 3 mm to protect the fish from jumping out of the tank. A constant photoperiod of 12 h light and 12 h dark was maintained and the light hours were between 7.00 am to 7.00 pm.

Formulation and Preparation of Experimental Diets

Fish meal (Clupeid), yellow maize, groundnut cake, soybean, blood meal, cassava starch, bone meal were purchased from Monday market in New-Bussa while vitamin/mineral premix was purchased from Rexton fisheries, Lagos and transported to New-Bussa for compounding of the diets. The earthworms used were cultured according to Sogbesan *et al.* (2007) and processed at the Fish Nutrition laboratory of National Institute for Freshwater Fisheries Research, New-Bussa using the method of Akpodiete and Okagbere (1999). Fishmeal, soybean meal (dehulled and toasted), groundnut cake, blood meal were used as dietary protein sources, palm oil was included as lipid source and cassava starch as a binder at a rate of 1.5%.

Five experimental diets coded D1 to D5 were formulated and earthworm meal was used in replacing fishmeal at various inclusion levels of 0% (control), 25, 50, 75 and 100% (Table 1).

Table 1: Formulation of ingredients (g/100 g of ingredients) in earthworm meal diets for the feeding trial

	Experimental diets				
Ingredients	D1 (Control)	D2	D3	D4	D5
Fish meal	30.0	22.5	15.0	7.5	0.0
Earthworm meal	0.0	7.5	15.0	22.5	30.0
Yellow maize	28.7	26.9	26.1	23.5	18.7
Groundnut cake	11.7	12.6	13.4	14.2	16.2
Soy bean meal	12.6	13.5	13.5	15.3	18.1
Blood meal	10.0	10.0	10.0	10.0	10.0
Vitamin/mineral premix*	2.0	2.0	2.0	2.0	2.0
Palm oil	2.0	2.0	2.0	2.0	2.0
Common salt	0.5	0.5	0.5	0.5	0.5
Bone meal	1.0	1.0	1.0	1.0	1.0
Cassava starch binder	1.5	1.5	1.5	1.5	1.5
Total	100.0	100.0	100.0	100.0	100.0
Calculated crude protein (%)	42.5	42.5	42.5	42.5	42.5
Calculated gross energy (kJ/100 g)	1750.0	1750.0	1750.0	1750.0	1750.0
Inclusion levels of earthworm meal (%)	0.0	25.0	50.0	75.0	100.0

*: 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; B2-6000mg; 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

All diets were isonitrogenous and isocaloric at 42.5% crude protein and 1900 kJ/100 g, respectively with the same P: GE of 22.4 mg of Protein kJ^{-1} of GE.

After formulation, the ingredients were measured using electric sensitive weighing balance (OHAUS- LS 2000 Model), milled into fine particulate (Falayi, 2003) using hammer machine. The milled ingredients were thoroughly mixed for 30 min to ensure homogeneity of the ingredients and pelleted wet using hand pelleting machine (Kitchen hand Cranker pelletizer). The pellets were collected in flat trays and sun-dried to constant weight after which the feeds were crushed into crumbs with pestle and mortar; then stored in jute bags at room temperature.

Experimental Fish

A total number of 250 fingerlings of *H. longifilis* of weight range 2.35-3.03 g with mean value of 2.68±0.29 g and total length range of 6.5-7.1 cm with mean value of 6.8±0.02 cm purchased from the Hatchery unit of NIFFR were acclimatized for one week in plastic holding tanks of 2.0×0.5×0.4 m aerated with Erckman electric aerator and fed by compounded NIFFR feed of 35% crude protein in the Fish Nutrition Laboratory of the Aquaculture and Biotechnology Department of NIFFR, New- Bussa.

Fingerlings were starved overnight, sorted, weighed and randomly stocked into the experimental tanks at the rate of 15 fingerlings per tank. The tanks were monitored for fish mortality daily. Dead fish were removed, counted and recorded for determination of survival rate.

Feeding of the Experimental Fish

Fish were offered 5% of their body weight meal per day; administered in two equal portions at 8.00-9.00 and 18.00-19.00 h. The quantity of feed was adjusted based on the weight of fish for previous week throughout the 8 weeks duration of the feeding trials.

Striking time is the time it takes the first fish to strike the first particle of the feed and is monitored immediately the feed is dropped using stop watch and recorded in seconds. The feed acceptability was estimated as the reciprocal of striking time following the method described by Eyo and Ezechie (2004).

Sampling of the Experimental Fish

The length and weight of each fingerling in each tank was measured at the commencement of the experiment. Subsequently, 5 fingerlings were taken randomly from each tank once a week and weighed with beam balance to determine the growth rates. At the end of this period five fish from each experimental diet were sampled for histometry of the liver, heart and visceral following Haruna (1997) method.

Water Quality Parameters

Water temperature record was taken daily before feeding at 7.00 -8.00 am with mercury-in-glass thermometer. Dissolved oxygen was monitored weekly using Winklers' method as described by Boyd (1983). pH was monitored weekly using a pH meter (Behrotest model).

Proximate and Energy Compositions of Experimental Diets and Fish Carcass

The proximate compositions of experimental diets and fish carcass before and after experiment were carried out at NIFFR chemical laboratory. They were analysed for crude protein, crude fibre, crude lipid, ash, Nitrogen free extracts, mineral salts, gross energy and digestible energy according to Association of Analytical Chemist Methods (AOAC, 2000).

Determination of Growth Performances, Feed Utilization, Histomerty and Economic Evaluations

The weekly weights recorded and feed supplied were used to compute the growth performances, nutrient utilization and economic evaluation indices following the methods of Oliva-Teles and Goncalves (2001) as:

Mean weight gain	=	W_f-W_i/n.
Relative growth rate	=	(Weight gain/Initial body weight) × 100.
Specific growth rate	=	(Log w_f-Log w_I/t) × 100.
Daily growth index	=	100 x $\{(w_f)^{1/3}$-$(w_I)^{1/3}/t\}$
Food conversion ratio	=	Feed intake (g)/Fish weight gain (g).
Protein efficiency rate	=	Weight gain/Protein intake
Hepato Somatic Index (HSI)	=	(liver weight/body weight) × 100.
Cardio Somatic Index (CSI)	=	(Heart weight/body weight) × 100.
Visceral Somatic Index (VSI)	=	(visceral weight/body weight) × 100

Feed production cost was based on the current market price of the ingredients from Rexton feed miller and Fish feed ingredient market at New-Bussa, Nigeria. The economic evaluations of the diets were calculated from the methods of Faturoti and Lawal (1986) and New (1989) as:

Investment cost	=	Cost of feeding (₦) + Cost of fingerlings stocked (₦).
Profit index	=	Value of fish (₦)/Cost of feed (₦)
Incidence of cost	=	Cost of feed (₦)/Mean weight gain of fish produced (g)
Benefit Cost Ratio (BCR)	=	Total cost of fish cropped (₦)/Total expenditure (₦)

Statistical Analysis of Data

All data collected were subjected to single analysis of variance (ANOVA) and Duncan Multiple test. Least Significance Differences (LSD) was used to determine the level of significance among treatments. Correlation and regression was carried out to determine the relationship between the treatments and some of the parameters using Graph pad Instat (DATASET1) computer package of Windows 2000.

RESULTS

The crude protein content of the five experimental diets were 43.53, 43.44, 43.32, 43.18 and 43.59% for 0, 25, 50, 75 and 100% earthworm meal proportion diet, respectively (Table 2). The highest crude lipid, 11.21% was in 100% earthworm meal based diet while lowest crude lipid, 10.42% was in 25% earthworm meal based diet. The gross energy values, which are 1776, 1783, 1792, 1803 and 1813 kJ/100 g increased as the earthworm meal inclusion increased from the control diet to 100% earthworm meal inclusion diet, respectively. A gradual rise in the line graph of D1 from week 0 till week 6 when there was a slight decrease and rise again in week 7 till the end of the experimental period. A gradual increase and rise in the slope of graph line D2 was recorded and shown as in Fig. 1. The growth pattern recorded in D5 slowly increased from week 1 till the end of the experiment. Highest mean weight gain of 6.45 g fish^{-1} was recorded in D2 while the lowest of 2.75 g fish^{-1} was recorded in D5. The highest SGR, 0.9507% was recorded in D2 while the lowest SGR, 0.5770% was recorded in D5. All growth indices were significantly different (p = 0.05). The FCR 1.51, 1.73, 1.79,

Table 2: Proximate composition (% day matter) and production cost (₦/kg) of the experimental diets

Compositions	D1	D2	D3	D4	D5
Dry matter (%)	84.28	84.41	84.53	84.56	84.83
Crude protein	43.53[a]	43.44[a]	43.32[a]	43.18[a]	43.59[a]
Crude lipid (%)	10.63[b]	10.42[b]	10.65[b]	11.09[a]	11.21[a]
Crude fibre (%)	3.36	3.51	3.67	3.82	4.03
Ash (%)	8.42	7.65	6.68	6.08	5.38
Nitrogen free extract (%)	18.33[c]	19.39[b]	20.03[a]	20.39[a]	20.62[a]
Sodium (g)	0.53	0.49	0.46	0.42	0.38
Calcium (g)	1.47	1.25	1.03	0.81	0.59
Potassium (g)	0.72	0.71	0.69	0.69	0.71
Phosphorus (g)	0.95	0.85	0.74	0.64	0.55
Gross energy (kJ/100 g)	1776.00[a]	1783.00[a]	1792.00[a]	1803.00[b]	1813.00[b]
P:GE	2.45	2.43	2.42	23.95	2.40
Digestible energy (kJ/100 g)**	240.00	241.00	242.00	244.00	247.00
Production cost (₦/100 g)	82.29	77.73	69.37	63.74	56.32

All values on the same row with the different superscripts are significantly difference $p<0.05$. **: Calculated as CP×23.06×0.9 + CL×39.5×0.85 + NFE×17.2×0.5 according to Morais *et al.* (2001)

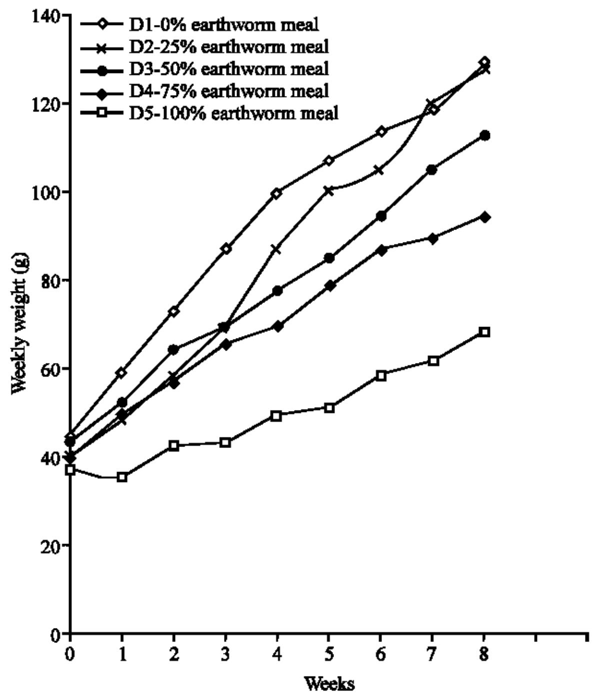

Fig. 1: Weekly increases in the weight of *H. longifilis* fingerlings to earthworm meal based diets for 56 dyas

2.06 and 2.59 were recorded for D2, D1, D3, D4 and D5. D2 had the highest PER of 1.52 while D5 had the lowest PER, 0.88 (Table 3). The highest gross energy retention of 1.04 was recorded in D4 while the lowest of 0.50 was recorded in the control diet. Highest daily energy gain of 3.34 kJ $fish^{-1}$ was recorded in control diet followed by 3.01 kJ $fish^{-1}$ from D2. There was significant different ($p = 0.03$) among the indices calculated for each treatment. The indices values determined in the control treatment were significantly higher than those from D5. The highest correlation $R = 0.996832$.

Table 3: Growth performances, feed efficiency and economic benefits of *H. longifilis* fingerlings fed earthworm meal diets for 56 days

Indices	D1	D2	D3	D4	D5	±SD
Mean initial weight (g fish^{-1})	2.96^{a}	2.68^{b}	2.90^{a}	2.65^{b}	2.49^{c}	0.19
Total initial weight (g)	44.40^{a}	40.20^{b}	43.35^{a}	39.75^{b}	37.32^{c}	2.86
Mean final weight (g fish^{-1})	9.23^{a}	9.13^{a}	8.05^{b}	7.27^{c}	5.24^{d}	1.64
Total final weight (g)	129.19^{a}	127.83^{a}	112.76^{b}	94.48^{c}	68.13^{d}	25.62
Mean weight gain (g fish^{-1})	6.27^{a}	6.45^{a}	5.15^{b}	4.62^{c}	2.75^{d}	1.49
Relative growth rate (% fish^{-1})	211.82^{b}	240.67^{a}	177.59^{c}	174.34^{c}	110.44^{e}	48.80
Mean weekly weight gain (g week^{-1})	10.60^{b}	10.95^{a}	8.66^{c}	6.84^{d}	3.85^{e}	2.93
Specific growth rate (% day^{-1})	0.882^{b}	0.951^{a}	0.792^{c}	0.783^{d}	0.577^{e}	0.14
Survival (%)	93.30^{a}	93.30^{a}	93.30^{a}	86.70^{b}	86.70^{b}	3.61
Striking time (sec)	4.10^{e}	6.10^{d}	7.30^{c}	8.90^{b}	10.80^{a}	2.57
Acceptability index (% sec^{-1})	24.39^{a}	16.39^{b}	13.70^{c}	11.24^{d}	9.26^{e}	5.89
Daily feed intake (g day^{-1})	2.63^{a}	2.36^{b}	2.22^{c}	2.01^{d}	1.42^{e}	0.46
Feed conversion rate	1.73^{c}	1.51^{d}	1.79^{c}	2.06^{b}	2.59^{a}	0.41
Protein efficiency rate	1.33^{b}	1.52^{a}	1.29^{b}	1.12^{c}	0.88^{d}	0.24
Apparent net protein utilization (%)	39.92^{c}	52.48^{a}	47.37^{b}	37.96^{c}	33.76^{d}	7.53
Apparent net lipid utilization (%)	20.87^{d}	24.75^{c}	33.67^{b}	34.91^{b}	44.87^{a}	9.40
Daily energy gain (kJ fish^{-1} day^{-1})	3.34^{a}	3.01^{ab}	2.84^{b}	2.79^{b}	1.98^{c}	0.50
Gross energy retention	0.50^{c}	0.59^{c}	0.58^{c}	0.84^{b}	1.04^{a}	0.22
Expenditure (₦)	421.30^{a}	402.67^{b}	386.16^{c}	371.78^{d}	344.86^{e}	29.23
Total sales (₦)	958.73^{a}	872.91^{b}	777.66^{c}	713.06^{d}	547.67^{e}	157.21
Profit Index	7.20^{e}	9.33^{c}	9.03^{d}	9.93^{b}	12.21^{a}	1.81
Incidence of cost	1.43^{b}	1.17^{e}	1.24^{d}	1.31^{c}	1.83^{a}	0.26
Benefit Cost Ratio (BCR)	2.07^{b}	2.38^{a}	2.01^{bc}	1.92^{c}	1.59^{d}	0.28

All data with superscripts are significantly different ($p<0.05$)

Table 4: Correlation matrices (r) of the evaluation indices of the fish response to the experimental diets

Indices	MWG	RGR	SGR	SR	AI	FCR	PER	ANPU	DEG	GER	PI	IC	BCR
MWG	1												
RGR	0.980317	1											
SGR	0.985482	0.996832	1										
SR	0.832587	0.759147	0.757308	1									
AI	0.797510	0.701516	0.702194	0.735272	1								
FCR	-0.975860	-0.966280	-0.974290	-0.856040	-0.670630	1							
PER	0.962364	0.970058	0.966577	0.863711	0.649095	-0.988960	1						
ANPU	0.741268	0.781303	0.772112	0.780546	0.284840	-0.857240	0.893506	1					
DEG	0.948061	0.887630	0.912417	0.739580	0.849187	-0.892610	0.837034	0.535459	1				
GER	-0.933940	-0.852930	-0.870200	-0.936170	-0.823430	0.927871	-0.894620	-0.705180	-0.922400	1			
PI	-0.880280	-0.776540	-0.805930	-0.772940	-0.901480	0.813952	-0.748030	-0.434540	-0.969800	0.934389	1		
IC	-0.802540	-0.823770	-0.852000	-0.608760	-0.318690	0.889762	-0.850640	-0.827860	-0.744520	0.736091	0.621817	1	
BCR	0.938365	0.978191	0.970426	0.766924	0.570699	-0.965640	0.984776	0.887149	0.795515	-0.812270	-0.668510	-0.86262	1

MWG: Mean Weight Gain; RGR: Relative Growth Rate; SGR: Specific Growth Rate; SR: Survival Rate; AI: Acceptance Index; FCR: Feed Conversion Ratio; PER: Protein Efficiency Rate; ANPU: Apparent Net Protein Utilization; DEG: Daily Energy Gain; GER: Gross Energy Retention; PI: Profit Index; BCR: Benefit Cost ratio

($p<0.05$) was recorded between relative weight gain and specific growth rate while the lowest R = -0.31869 ($p<0.05$) was recorded between acceptability index and incidence of cost (Table 4).

DISCUSSION

The fact that weekly weight increase was recorded in all the treatments showed that none of the experimental diets contain anti-growth factors (Sogbesan *et al*., 2006). In the present study, the proportion of dry earthworm meal appeared to be an important factor in influencing growth, acceptability index, feed conversion ratio, protein efficiency value, energy retention and daily energy gain and the cost benefit values of the feeding *H. longifilis* than each index determined. This observation was in agreement with the report Stafford and Tacon (1985) that weight gain reduces as the inclusion levels of earthworm increases in practical diets fed to rainbrow trout. Similar report was made by Sayed (1999) who recorded that adverse effect on the productivity of tilapia when fed earthworm supplemented diets. Differences in the quality of protein fed to the fish as shown from the result of weight gain and protein efficiency rate may have been influenced by the digestibility, indispensable amino acid composition, availability (biological value), palatability and presence of toxic compounds (Scott *et al*., 1976) in the experimental diets. The Apparent Net Protein Utilization (ANPU) was highly significant $R = 0.893506$; $p<0.05$ with protein efficiency rate. This 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 dry earthworm meal at the lowest treatment level showed a better ANPU compared to both the control and other (different inclusion levels) may indicate that earthworm contain some proteolitic factors which limits its utilization at higher inclusion levels (Stafford and Tacon, 1985). The reduction in the acceptability index reported from this study could have been as a result of secretion of eleocytic cells in the epithelium. This similar observation was made by Alegbeleye and Oresegun (1998) when they substituted three lumbrid worms namely *Eisena foetida*, *Dendrobaena veneta* and *Dendrobaena subrubicunda* for trash fish (on weight basis) as protein sources in *Oreochromis niloticus* feed. Their results showed that *E. foetida* fed fish had the best performance in terms of overall growth and nutrient utilization efficiency in comparison with those fed the other lumbricid diets. However, the fish fed trash fish showed a significantly higher ($p<0.05$) feed intake, protein intake and protein deposition over all the lumbricid worm diets. The authors linked the poor growth and nutrient utilization of *O. niloticus* fed complete earthworm meal to unpalatable tendency of the worms due to secretion of eleocyte cells in its epithelium. Despite this, Cardinete *et al*. (1991) had also reported reduction in food intake and nutritive utilization of protein in rainbow trout fingerlings fed earthworm meal diet. They as well mentioned that the reduction was as a result of the ceolmic fluid present in the earthworm. This fluid was also reported to be responsible for reduction of proteolitic activity in the digestive tract of fish fed. This affirmed the inhibitory effect of the fluid on digestive enzymes activity in fish.

The substitution of dry earthworm meal for fish meal lowers the cost of diet production (Table 1) which is an indication of a more cost efficient and cheaper non-conventional ingredient in relative to the fish meal. Consequently, the farmer will benefit economically through the utilization of this cheaper ingredient (at 25% inclusion) to raise *H. longifilis*.

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