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Amaranthus spinosus Leaf Meal as Potential Dietary Protein Source in the Practical Diets for *Clarias gariepinus* (Burchell, 1822) *Fingerlings*

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ABSTRACT

The aim of this study was to evaluate the potentials of *Amaranthus spinosus* leaf meal as dietary protein source for *Clarias gariepinus* fingerlings. An 8 week feeding trial was conducted in plastic aquaria tanks of 50 L capacity. *Amaranthus spinosus* leaf meal was included in the practical diets at 0, 5, 10, 15 and 20% designated as diets 1, 2, 3, 4 and 5, respectively. Diet 1 without *A. spinosus* serves as the control. All diets were made isonitrogenous (36% CP) and isocaloric. Fingerlings of initial mean weight of 5.00±0.37 g were fed on allotted diet at 3% b.wt. day^{-1} for 56 days. Specific Growth Rate (SGR) was highest with a value of 1.95±0.69 in diet 1 while it was lowest in diet 5 with a value of 0.20±0.24, SGR values in diet 1 (control) and diet 2 were similar and significantly ($p<0.05$) better than the other dietary treatments. Fish fed diets 3, 4 and 5 showed significantly reduced growth performance and feed utilization compared to those fed with diets 1 and 2. FCR was lowest in fish fed diet 1 with a value of 1.72±0.56 and highest in fish fed with diet 5, however, FCR values of diets 1 and 2 were not significantly ($p>0.05$) different from each other but were significantly ($p<0.05$) different from other diets. This study indicates that up to 5% *A. spinosus* leaf meal could be included in the practical diet of Clarias *gariepinus* without affecting growth and feed utilization.

Key words: *Amaranthus spinosus*, growth performance, feed utilization, dietary protein, *Clarias gariepinus*

INTRODUCTION

The African catfish, *Clarias gariepinus* is one of the popular fish cultured in Africa because of its fast growth, disease resistance, hardiness, excellent taste and high market demand (Adewolu *et al*., 2008, 2009). However, one of the problems hindering the successful and large scale production of this fish is the high cost of feed. This has been attributed to the fact that most protein ingredients, that are used for fish feed are also used for livestock feed and for human consumption, making them to be scarce and expensive. It is therefore important to search for alternative fish feed ingredient of high nutritional value that are cheap, available and not in competitive for human, livestock or industrial uses, these ingredients according to De Silva and Anderson (1995) are referred to as unconventional ingredients.

Leaf meal proteins are among the unconventional sources of protein that may reduce the high cost of fish feed (De Silva and Anderson, 1995). A particular leaf meal of interest as a potential dietary protein source in fish feed is *A. spinosus*.

A. spinosus belongs to the family Amaranthecae. It is an annual plant found in tropical regions of America, Africa and Asia (Steentoft, 1988). They are widely available during the raining season and grow mostly on every soil and thus, regarded as weed (Grubben and Denton, 2004).

The leaves of this plant are not edible by man or livestock due to the presence of thistles on their stems. It has not been used for agricultural and industrial purposes in Nigeria thus, making this plant to be abundant with little or no cost. The chemical analysis of this plant show that it is high in protein (30-32%) with lysine constituting as much as 5.9% which is equal to the amount found in soybean and more than that present in some of the best maize strains (Oyenuga, 1968; Tindall, 1983; Steentoft, 1988; Adeniji *et al*., 2007; Emokaro and Ekunwe, 2007). This plant could therefore, form a valuable potential feed ingredient for aquafeed.

The inclusion of leafmeal in aquaculture feed is fast gaining global attention over the years because of its availability, protein and mineral/vitamin contents and economic feasibility (Tacon, 1997; El-Sayed, 1999; Ali *et al.,* 2003). Several studies had been conducted on the use of terrestrial and aquatic leafmeals as dietary protein sources in fish feed. These include (Reyes and Fermin, 2003) on *Carica papaya* leaf meal; leuceana leaf meal (Bairagi *et al*., 2004). Ali *et al.* (2003) on Alfalfa leaf meal; Cassava leaf meal (Bureau and De la Noue, 1995; Madalla, 2008); Moringa leaf meal (Madalla, 2008) potato leaf meal (Adewolu, 2008).

There is paucity of information on the use of *A. spinosus* leaf meal as a potential protein ingredient in the practical diets of *Clarias gariepinus,* a culturable omnivorous fish species that can utilize both animal and plant protein well. The aim of this study, therefore, was to assess the suitability of *A. spinosus* as dietary protein ingredient in practical diets of fingerlings of *Clarias gariepinus*.

MATERIALS AND METHODS

Collection and preparation of ingredients: Fresh leaves of *A.spinosus* were collected from Badagry Lagos State Nigeria in April 2009. The leaves were washed with tap water to remove dirt and other debris drained properly and sun-dried to a constant weight. These were ground with a kitchen blender to powdered form, packed and kept in air tight covered bottle until needed.

Diet formulation and preparation: Five isonitrogenous and isocaloric diets were formulated using Pearson Square method as described by Gohl (1985) to contain 36% crude protein. *Amaranthus spinosus* leaf meal was incorporated into each of these diets at 0, 5, 10, 15 and 20% designated as diets 1, 2, 3, 4 and 5, respectively to replace other protein ingredients in the diets. The diet containing 0% leaf meal served as the control. Feed ingredients were weighed according to the formulation composition shown in Table 1. The feed ingredients were mixed using a kitchen mixer before the addition of vitamin premix. Vegetable oil was added to the dry ingredients and then mixed thoroughly. Leaf meal was added to the premixed feed ingredients mixed again, warm water was added to the mixture and homogenized until a dough-like paste was formed. The dough was passed through an improvised pelleting machine with a 1.5 mm die. The moist pellets were oven dried at 60°C to a constant weight, cooled at room temperature, stored in labeled air tight containers.

Experimental design and feeding trials: Fingerlings of *Clarias gariepinus* of mean body weight of 5.00±0.37 g were randomly stocked at 20 fish per tank into 18 flow-through plastic aquaria tanks of 50 L in capacity. Each of the diet was randomly assigned to three replicate tanks in a completely

Table 1: Percentage gross composition of the experimental diets containing 0,5,10, 15 and 20% *A. spinosus* leaf meal (Diet 1, 2, 3, 4 and 5, respectively)

Feed ingredients	% Crude protein	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal	72	27.35	27.35	27.15	26.00	25.00
Soy meal cake	42	27.35	25.00	22.00	20.00	18.00
Maize	9.0	42.53	39.90	38.20	36.25	34.25
A. spinosus	31.9	-	0.50	10.00	15.00	20.00
Premix*		0.50	0.50	0.50	0.50	0.50
Dicalcum PO_4		0.25	0.25	0.25	0.25	0.25
Groundnut oil		2.00	2.00	2.00	2.00	2.00
Grass energy kcal kg^{-1}	-	4507.85	4439.79	4372.37	4272.93	4221.36
Digestible energy kcal kg^{-1}	-	2924.76	2880.90	28365.77	2787.54	2740.50

NFE (Nitrogen free extract) = 100 (Crude protein+Crude Lipid+Crude Fibre+Total Ash). Gross energy = Caloric value of protein 5.65, NFE 4.1 and lipid 9.45 kcal kg^{-1} (Brett, 1973) Digestible energy = Caloric value of protein 3.5, NFE 2.5 and (Lipid) 8.1 kcal kg^{-1} (NRC, 1993). *Composition of vitamin premix, Each kg of the diet contained 2,000,000 IU vit. A: 4,000,000 IU vit. D3; 200,000 vit. E: 1,200 mg vit. K: 10,000 mg vit. B1: 30,000 mg vit. B2: 19,000 mg vit. B6: 1000 mg vit. B12: 5000 mg, Panthotenic acid: 200,000 mg, Niacin: 5,000 mg Folic acid: 30 g Mn; 40 g Zn; 40 mg Fe; 4 g Cu; 5 g I_2; 0.2 mg Co; 600 g calcium; 400 mg choline chloride; 40 mg biotin; 400,000 mg phosphorus; 100,000 mg lysine and 400 g methionine

randomized design. Fish were allowed to acclimatize for 7 days to experimental conditions, during this period they were fed with commercial diet. Prior to the commencement of the feeding trial, all fish were starved for 24 h. This practice was to eliminate variation in weight due to residue food in the gut and also to prepare the gastro intestinal tract of fish for the experimental diets, while at the same time to increase the appetite of fish. Fish were fed with allotted experimental diets at 3% of their total body weight per day. Total ration was divided into two feedings: one half was given at 09:00 h and the remaining half was given at 17:00 h except on weighing days when they were fed after weighing. All fish were reweighed every two weeks and feed weight was adjusted accordingly to accommodate for weight changes. The feeding trial lasted for 56 days, between April and June 2009.

Chemical analysis: Samples of *A. spinosus*, leaf meal, other feed ingredients, experimental diets and experimental fish were subjected to proximate analysis. Moisture was obtained by drying the sample at 105°C in an oven until constant weight was obtained. Crude protein was determined by using the microkjeldah digestion method (Nx6.25). Crude lipid by soxhlet-extraction method. Ash content by combustion in muffle furnance to constant weight at 600°C. Crude fiber was done by using the acid/base digestion process. Nitrogen free extract was calculated by taking the sum values for crude protein, crude lipid, crude fiber, total ash and moisture and subtracting these from 100. All analysis followed the procedures of AOAC (1995).

Water management and analysis: There was 50% exchange of water in all the tanks daily and continuous aeration was provided to each tank through air stones connected to air compressor. Water temperature, pH, dissolved oxygen and ammonia concentrations in water were monitored everyday except ammonia which was monitored once a week. Temperature was measured using a mercury glass thermometer. pH was measured with a pH meter (Jenway model 9060) dissolved oxygen with an oxygen meter (Hanna model H1-9142) while ammonia was determined in the laboratory according to APHA (1985). The water temperature varied between 26-280C, pH ranged from 6.5 to 7.5, dissolved oxygen levels varied from 4.5-5.5 mg L^{-1} while ammonia concentration in water was between 0.03-0.05 mg L^{-1} throughout the experimental period.

Evaluation of growth and feed utilization parameters: Mean weight gain (WTG), Specific Growth Rate (SGR), Percentage Weight Gain (PWG), Protein Efficiency Ratio (PER), Feed Intake (FI), Protein Intake (PI) and Feed Conversion Ratio (FCR) were calculated according to the following:

$$\text{WTG} = \text{Mean final body weight-mean initial body weight} \quad (1)$$

$$\text{PWG} = \frac{\text{Mean weight gain}}{\text{Mean initial weight}} \times 100 \quad (2)$$

$$\text{SGR}\left(\frac{\%\text{BW}}{\text{day}}\right) = \frac{(\text{logeWf-logeWi}) \times 100}{\text{T}} \quad (3)$$

where, T represents trial duration (day Wf and Wi represent mean final and initial weights (g), respectively.

$$\text{FCR} = \frac{\text{Weight of dry feed fed (g)}}{\text{Weight gain of fish (g)}} \quad (4)$$

$$\text{PER} = \frac{\text{Gain in weight of fish (g)}}{\text{Protein intake (PI) (g)}} \quad (5)$$

$$\text{P1} = \text{Feed intake (FI) X \% protein in diet} \quad (6)$$

$$\text{F1} = 3\% \text{ Body weight of fish per day} \quad (7)$$

$$\text{Survival (\%)} = \frac{\text{-S1}}{\text{S2}} \times 100 \quad (8)$$

Where:
S1 = No. of fish at the end of experiment
S2 = No. of fish at the beginning of experiment

Statistical analysis: All data gathered after the feeding trial were analyzed by one-way Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test to test for significant differences among treatments. Analysis was performed using the SPSS version II (Statistical Package for Social Sciences Version II). Significant level was chosen at $p<0.05$. Values were expressed as Means±SD.

RESULTS

Composition of feed ingredient and experimental diets: The results of proximate composition of *A. spinosus* leaf meal and other feed ingredients are presented in Table 2, the crude protein content of *A. spinosus* leaf meal was 31.9%, crude lipid 3.7%, crude fiber 9.8% and total ash 15.1%. Proximate composition of the experimental diets is shown in Table 3. There were very little

Table 2: Nutrient composition of feed ingredients

Feed ingredient	Dry matter	Crude protein	Crude lipid	Crude fibre	Total ash	NFE	Gross energy	Digestible energy
							----------(kcal kg^{-1})----------	
Fish meal	91.50	72.0	3.0	1.50	15.0	8.50	4700.0	2975.5
Soybean	87.50	42.0	8.0	8.50	33.5	33.51	3502.5	2955.5
Maize	90.45	9.0	4.0	1.35	3.9	81.75	4238.3	2682.5
A. spinosus	19.40	31.9	3.7	9.80	15.1	20.10	2976.1	1918.7

NFE (Nitrogen free extract) = 100-(Crude protein+crude lipid+crude fibre+total ash). Gross energy = Caloric value of protein 5.65, NFE 4.1 and lipid 9.45 kcal kg^{-1} (Brett, 1973). Digestible energy = Caloric value of protein 3.5, NFE 2.5 and (Lipid) 8.1 kcal kg^{-1} (NRC, 1993)

Table 3: Proximate compositions of experimental diets containing 0, 5, 10, 15 and 20% *A.spinosus* leaf meal (Diet 1, 2, 3, 4 and 5, respectively)

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fiber	3.40	3.60	3.80	4.10	4.35
Ash	8.00	8.50	8.70	9.20	9.60
Fat	6.80	6.70	6.50	6.40	6.30
Protein	36.00	35.80	35.85	35.68	34.98
NFE	45.80	42.40	45.15	44.62	44.97
Digestible energy kcal kg^{-1}	2955.80	2855.70	2910.00	2882.87	2853.85
Gross energy kcal kg^{-1}	4554.40	4394.25	4490.93	4458.14	4407.29

NFE: Nitrogen free extract, NFE: 100 (Crude protein+crude lipid+crude fibre+total ash), Gross energy = Caloric value of protein 5.65, NFE 4.1 and lipid 9.45 kcal kg^{-1} (Brett, 1973). Digestible energy = Caloric value of protein 3.5, NFE 2.5 and (Lipid) 8.1 kcal kg^{-1} (NRC, 1993)

variations in the nutrient content of various experiment diets. The protein content ranged from 34.98-36.002 and gross energy from 4407.29-4554.4 kcal kg^{-1}.

General observations: Fish in all dietary treatments consumed their allotted experimental diets. There was no rejection of feed. However, towards the end of the experiment, fish fed with diet 5 (20% leaf meal) consumed their diet reluctantly. There were no signs of diseases observed in any of the dietary group.

Growth and feed utilization of fish: The growth performance and feed utilization of *Clarias gariepinus* fingerlings in terms of weight gain (WTG), Percentage Weight Gain (PWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) are presented in Table 4. The mean final weight of fish increased from the initial values in all the dietary treatments. *Clarias gariepinus* fingerlings fed with the control diet (diet 1) had the highest weight gain while diet 5 had the poorest weight gain. The general trend was that decreasing growth rate was observed with increasing inclusion level of *A. spinosus* leaf meal in experimental diets. However, there were no significant ($p>0.05$) differences in weight gain of fingerlings fed Diets 1 and 2. Fish fed diets 3, 4 and 5 containing 10, 15 and 20% of *A. spinosus* leaf meal showed significantly ($p<0.05$) reduced growth performance compared to those fed diets 1 and 2. The growth performance of fish fed diets 3, 4 and 5 were significantly ($p<0.05$) different from each other. These trends were observed for SGR, PWG.

The FCR was lowest in fish fed diet 1 with a value of 1.72±0.56 and highest in fish fed with diet 5, however, FCR values of diets 1 and 2 were not significantly ($p>0.05$) different from each other but were significantly ($p<0.05$) different from other diets.

Table 4: Growth response and feed utilization of *Clarias gariepinus* fed diets containing 0, 5, 10, 15 and 20% *A. spinosus* leaf meal (Diet 1, 2, 3, 4 and 5, respectively)

Performance indices	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Initial weight (g)	4.88±0.20	5.20±0.40	5.23±0.18	5.17±0.14	5.28±0.18
Final weight (g)	15.27±5.36d	14.44±0.80d	10.28±1.17c	7.06±0.41b	5.96±0.97a
Weight gain (g)	10.39±5.29d	9.25±1.18d	5.05±1.27c	1.89±0.29?	0.68±0.87a
Percentage weight gain	211.99±62.57d	180.22±38.04d	96.85±26.37c	36.42±4.6b	12.71±15.93a
Specific growth rate	1.95±0.69d	1.83±0.23d	1.20±0.25c	0.55±0.06b	0.20±0.24a
Feed intake (g)	15.89±4.77c	16.03±1.46c	12.91±1.14b	10.65±1.70a	9.94±0.43a
Protein intake (g)	5.56±1.67c	5.62±0.51c	4.52±0.40b	3.73±0.59a	3.48±0.15a
Feed conversion ratio	1.72±0.56d	1.74±0.08d	2.65±0.59c	5.66±0.62b	66.34±8.33a
Protein efficiency ratio	1.77±0.49c	1.64±0.07c	1.11±0.22b	0.51±0.06a	0.19±0.23a
Survival (%)	83.33±10.41c	70.00±5.00 b	61.67±10.41a	66.67±2.89b	61.67±2.89a

Values in the same row having different superscripts are significantly different ($p<0.05$) and values in the same row with no superscript are not significantly different ($p>0.05$)

Table 5: Proximate body composition of fish fed experimental diets containing 0, 5, 10, 15 and 20% *A. spinosus* leaf meal (Diet 1, 2, 3, 4 and 5, respectively)

		Final				
Components	Initial fish	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Moisture	79.50	76.0±0.05a	76.20±0.04a	76.90±0.2b	77.50±0.20c	77.95±0.2d
Crude protein	13.80	15.0±0.05a	15.00±0.06a	14.50±0.10b	14.40±0.11c	14.00±0.08c
Crude lipid	4.00	5.80±0.03a	5.80±0.02a	5.20±0.01b	4.80±0.04c	4.20±0.01d
Ash	2.40	2.50±0.06	2.50±0.05	2.70±0.10	2.80±0.11	2.90±0.10

Values in the same row having different superscripts are significantly different ($p<0.05$) and values in the same row with no superscript are not significantly different ($p>0.05$)

The PER values of fish fed experiment diets ranged from 1.77±0.4 in diet 1 to 0.19±0.23 in diet 5. The values recorded for fish fed diets 1 and 2 were not significantly affected by the level of inclusion of *A. spinosus* leaf meal. However, in the other diets, the levels of *A. spinosus* at 10, 15 and 20% inclusion levels significantly affected the PER values. The percentage survivals of experimental fish was high at lower inclusion of leaf meal (diets 1 and 2) above 70% and were below 70% at higher inclusion of leaf meal in diets 3, 4 and 5.

The results of carcass composition at the start and at the end of the experiment are presented in Table 5, fish fed the control diet and diet 2 (5% leaf meal) had significantly higher body crude protein and crude fat than fish fed with other diets. Fish fed diets 3, 4 and 5 had significantly higher whole body moisture and lower lipid content than fish fed with diets 1 and 2. There were no significant differences in the total ash content of fish fed with different diets.

DISCUSSION

The potentials of a feed ingredient such as leaf meal in fish diets can be assessed on the basis of its chemical composition. The proximate composition of *A. spinosus* leaf meal in this study showed that the crude protein content was 31.9%, crude lipid 3.7%, crude fibre 9.8% and total ash 15.1%. These values were higher than the values reported by Adeniji *et al.* (2007). The differences might, perhaps, be due to environmental conditions such as soil type, harvesting time, method of sampling and processing methods (Ravindran 1993; Madalla, 2008).

In the present investigation, all the experimental diets were accepted by *C. gariepinus* except towards the end of the experiment, where fish fed with diet 5 (20% leaf meal) consumed their diet reluctantly. This showed that the levels of incorporation of Amaranthus leaf meal in the diets were not likely to affect the acceptability of the diets by the fish, thus supporting the work of Francis *et al.* (2001), Siddhuraju and Becker (2003) and Adeniji *et al.* (2007).

Studies on the utilization of various leaf meals as dietary protein source have been conducted for Clarias gariepinus with variable results (Bureau and De la Noue, 1995; Olukunle and Agboola, 2005; Konyeme *et al.*, 2006). In the this investigation, the results of *Clarias gariepinus* fingerlings fed diets containing various levels of *A. spinosus* revealed that fish fed diet 2 containing 5% *A. spinosus* leaf meal had growth performance similar to fish fed the control diet. This result is different from the work of Adeniji *et al.* (2007) who fed fingerlings of *Oreochromis niloticus* with diets containing 25 to 75% *Amaranthus spinuous*. They reported reduced growth of fish at all levels of inclusion. The differences with the results in the present study might be due to the different percentages of inclusion of *A. spinosus* leaf meal and different fish species.

The significantly better growth of fish fed with diet 2, containing 5% *A. spinosus* leaf meal might be due to the fact that the essential amino acid composition was well balanced in the diet and the levels of antinutritional factors were below the levels that might inhibit growth in *C. gariepinus*. This finding, therefore, indicates that up to 5% of *A. spinosus* leaf meal can be included in the practical diet of *C. gariepinus*.

To date, there is no published information on the incorporation of *A. spinosus* leaf meal in the diet of *C. gariepinus*. However, available information on other leaf meals revealed that a maximum of 10% cassava leaf meal could be incorporated in *C. gariepius* diets (Bureau and De la Noue, 1995). Olukunle and Agboola (2005) reported that 25% of duck weed leaf meal could be included in *C. gariepinus* diets. Recently, Konyeme *et al.* (2006) found that 40% level of water hyacinth leaf meal could be included in practical diets of *C. gariepinus* without affecting growth.

In this study, the reduced growth performance of fish fed with diets containing 10, 15 and 20% leaf meal might not be a palatability problem, because the diets were accepted by fish but might be related to the presence of various antinutritional factors. *A. spinosus* leaf meal has been reported to contain saponins, alkaloids, phenols and oxalates as ANFS- (Tindall, 1983; Bressani, 1994). Poor growth performance of diets containing these ANFs has been observed by Adeniji *et al.* (2007) in Nile Tilapia, *Oreochromis niloticus*. The contents of these antinutrients increased with increasing level inclusion levels of *A. spinosus* leaf meal hence resulting in reduced growth performance. The adverse effects of ANFs in fish have been reviewed by Francis *et al.* (2001). Saponins, alkaloids phenols and oxalates found in many plants are considered to be very toxic and growth deterrent in fish. These antinutrients inhibit protein and other nutrient digestion (Bressani, 1994; Bureau and De la Noue, 1995; Tindall, 1983; Francis *et al.*, 2001; Hossain *et al.*, 2001). Another reason for poor growth performance of fish fed diets containing levels above 5% inclusion of leaf meal could be as a result of imbalance of amino acids (Ogunji, 2004; Hossain *et al.*, 2001), especially methionine. Deficiency in methionine may lead to reduced fish growth.

Although, the crude fiber content of the experimental diets increased with the increasing level of Amaranthus leaf meal, these levels were within the recommended range of less than 5% for commercial catfish feed (Phonekhampheng, 2008). Therefore, the reduced growth performance of catfish may not be as a result of levels of crude fiber is present in the diets.

The proximate carcass composition data of *C. gariepinus* showed that fish fed the control diet and diet 2 (5% leaf meal) had significantly higher body crude protein and crude fat than fish fed

with other diets. This observation is in accordance with the reports of Hossain *et al.* (2001) and Madalla (2008). Diets containing higher levels of *Amaranthus spinious* produced significantly the highest body moisture and lowest body lipid. The reason here might be that fish tend to utilize body lipid to sustain metabolism when food energy is not sufficient because of the antinutrients that inhibit nutrient digestion. This is supported by Han *et al.* (2000) who reported that the presence of saponins may have contributed to inhibit pancreatic lipase activity thus, delayed intestinal absorption of dietary fat.

CONCLUSION

In conclusion, the results of the present study indicate that up to 5% of *A.spinosus* leaf meal can be included in the practical diet of *C. gariepinus* without affecting growth and feed utilization. Despite the low level of 5% performance, *A. spinosus* leaves still have the potential to serve as a cheap source of protein in Nigeria due to their abundance and non usage by either man or animals.

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