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Optimum Dietary Crude Protein and Digestible Energy Requirements of Clariid catfish *Clarias gariepinus* (Burchell, 1822) Fingerlings in the Tropics

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ABSTRACT: This study determined the optimum protein and digestible energy levels for *Clarias gariepinus* fingerlings fed local feed. Sixteen diets of four digestible energy (DE) level (2400, 2600, 2800 and 3000 Kcal/Kg) each at four crude proteins (CP) levels (25, 30, 35 and 40%) were formulated and fed to the fingerlings for 70days. The result showed that the highest final weight of fish (34.24g) was obtained in the 35 %CP (DE of 2400 kcal/kg). The FCR decreased with increase in DE with the values obtained in the diets containing 2800 and 3000 Kcal/kg diet significantly lower than that obtained from diet containing 2400 Kcal/kg. The Protein efficiency ratio decreased significantly with increase in CP up to 35 %CP and increased at 40 %CP. From the overall results, it can be concluded that the best body weight values (Weight gain, RWG and SGR), were obtained at protein levels of 35 and 40% and energy levels of 2600, 2800 and 3000 Kcal/kg. The best FCR were obtained at CP levels of 30% and 40% and DE of 2600, 2800 and 3000 Kcal/kg. Therefore, for *C. gariepinus* the optimum protein levels lies between 35 and 40% at the digestible energy value of 2600 or 2800 Kcal/kg.

Keywords: Catfish, Optimum energy, Protein levels, Fish diet

Introduction

The vast Nigerian aquatic medium of numerous water bodies like rivers, streams, lakes, reservoirs, flood plains, irrigation canals, coastal swamps offer great potentials for aquaculture production in Nigeria. Oladejo (2010) set to place a distinction on fish farming and aquaculture defining fish farming as the sub-set of aquaculture that focuses on rearing of fish under controlled or semi-controlled conditions for economic and social benefits while aquaculture as that which deals with the rearing of aquatic organisms (fishes, mollusc, crustaceans and aquatic plants) under controlled or semi-controlled conditions for economic and social benefits. In fish farming, nutrition is critical because feed represents 50 – 60% of total production cost (Ajonina and Nyambi, 2013). Fish requires high quality nutritionally balanced diet for growth and attainment of market size within the shortest possible time (Gabriel, 2007). For maximal growth, fish nutrition needs to be tailored to the species and the stage of development. Given that fish feed is one of the highest operating costs of an aquaculture system **(**FAO, 2006**)**, it is necessary to maximize the feed conversion ratio and use costly feed ingredients judiciously. Information on type, quality, quantity, seasonality and cost of fish feeds is important in determining the appropriate production strategy (Sogbesan *et al.*, 2009). Lack of readily available nutritive fish feed ingredients have continued to be a major constraint to the survival of aquaculture in the competitive global food production system (Ogunji *et al.*, 2005).

Materials and methods

This study was conducted in the Experimental fish farm of the Department of Fisheries, University of Benin, Benin City, Nigeria to ascertain the optimum protein and digestible energy levels for *Clarias gariepinus* fingerlings

Experimental Diets: Sixteen (16) diets were prepared for the feeding trials. The diets were formulated containing four digestible energy (DE) levels of 2400, 2600, 2800 and 3000Kcal/kg, each at four (4) crude protein levels of 25, 30, 35 and 40%. The layout of the dietary treatment is shown in Table 1. Each diet constituted a treatment. The detail of nutrient composition of feedstuffs of experimental diets and proximate analysis is shown in Table 2. The levels of feed ingredients used to formulate the diets were manipulated to obtain the desired levels of DE and CP. Calculation of the DE levels of the diets were based on the cumulative of DE of the ingredients as recommended for channel catfish by Lovell (1984). For the crude protein, lysine and methionine, the various recommended by New (1987) were used. These values are shown Table 2.
In preparing the diets, ingredients were milled, mixed and prepared as described by Martinez-Palacios *et al*, (1996). The milled ingredients were sieved through standard sieve Nos. 16 and 20 (maximum of 1.19mm). The homogenous feed mixes were processed into pellets or granules (2 mm) with gelatinized corn starch component as the binder. After preparation, pelleted diets were oven-dried at 70^0c for 24 hours. Feed samples were stored in polythene bags in cupboard at laboratory temperature. Dried granules of feed samples were taken for proximate analysis. All ingredients were locally sourced for the trial conducted.

Table 1: Layout for dietary treatments

Digestible Energy (DE Kcal/Kg)	**Diets (% Crude protein)**			
	25%	**30%**	**35%**	**40%**
2400	2400(1)	2400(5)	2400(9)	2400(13)
2600	2600(2)	2600(6)	2600(10)	2600(14)
2800	2800(3)	2800(7)	2800(11)	2800(15)
3000	3000(4)	3000(8)	3000(12)	3000(16)

NB: Numbers in parenthesis represent the various treatment codes.

There were four trials, one trial for each type of feed. Glass tank was used for the trials. Each tank was connected to a central aerator. Water supplied by the university of Benin Campus domestic water services was maintained at 35 litre mark/level throughout the experiment. Fingerings were fed test diets twice daily during daylight (9:30 am and 4:00pm). At each time of feeding, animals were fed to satiation i.e. hand fed access to food, during which diet was provided in small amount at a time, so that the fish will eat nearly all the diet offered. Water temperature was measured twice daily during feeding. Dissolved oxygen (DO) was measured once a week using Winkler's method. Daily observations were made to detect any abnormality and fish mortality. Unconsumed diets and faecal wastes were removed by siphoning daily. Each trial lasted 70days. Weight of fish per treatment and per replicate was recorded weekly. Weight of food consumed by fish was also recorded weekly for each replicate. In order to obtain the weights of the fish, fish were batch weighted in a dish containing pre-weighed water.
Carcass Analysis: All the diets and carcasses were subjected to proximate analysis at the end of the trials. Crude protein (N X 6.25) was determined by the micro-kjeldahl method and crude fibre (CF) was by the system based on acid-alkaline digestion. Lipids, ash and moisture were determined using standard methods in triplicate.
Growth and Nutrient Utilization indices: Weights of fish and feed consumption were obtained at weekly intervals. From the fish weights and feed consumption, the following were determined:

Weight gain = $W_1 - W_0$ (g)

Relative Weight Gain (RWG %) = $(W1 - W0)/W0 \times 100$ (%)

Specific Growth Rate (SGR %) = $\frac{(\ln W1 - \ln W0)}{T} \times 100$ (%/week)

Where: W_0: mean initial weight (g)
W_1: mean final weight (g)
T: time in 7 days between weightings

Feed conversion ratio (FCR) = $\frac{\text{feed intake (g)}}{\text{wet weight gain}}$ (g)

Protein efficiency ratio (PER) = $\frac{\text{weight gain}}{\text{protein intake}}$

Net protein utilization (NPU) = $\frac{BP1 - BP0}{CP} \times 100$

Table 2: Ingredient composition and proximate analysis of experimental diets (%)

Ingredient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Maize	29.79	27.29	24.79	22.29	18.29	19.20	19.79	17.79	24.44	22.94	20.44	17.44	3.44	6.14	13.44	10.94
Fishmeal	7.00	7.00	7.00	7.00	14.50	14.50	14.50	14.50	21.40	21.40	21.40	21.40	26.40	26.40	26.40	26.40
Soybean meal	16.77	16.77	16.77	16.77	18.77	18.77	18.77	18.77	20.20	20.20	20.20	20.20	23.20	24.20	24.20	24.20
Brewers yeast	12.77	12.77	12.77	12.77	20.20	17.86	14.77	14.77	16.40	16.40	16.40	16.40	27.40	25.40	18.10	18.40
Wheat bran	27.58	27.58	27.58	27.58	23.60	23.08	22.58	22.58	14.10	14.10	14.10	14.10	16.10	14.40	14.40	14.10
Soybean oil	2.63	5.13	7.63	10.13	1.18	3.13	6.13	8.13	0.00	1.50	4.00	7.00	0.00	0.00	0.00	2.50
Bonemeal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vit. Premix	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin E	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Analyses																
DE calculated	2400	2600	2800	3000	2400	2600	2800	3000	2400	2600	2800	3000	2400	2600	2800	3000
CP calculated	25.0	25.0	25.0	25.0	30.0	30.0	30.0	30.0	35.0	35.0	35.0	35.0	40.0	40.0	40.0	40.0
CP Analysed	24.92	24.68	24.44	24.20	32.51	31.54	31.54	30.02	35.54	35.33	35.15	34.86	43.47	43.10	40.54	40.38
Moisture (%)	8.08	8.14	8.05	8.31	8.16	8.09	8.06	9.06	9.07	8.82	8.91	8.69	8.71	8.84	9.01	9.02
Lipid (%)	3.51	6.03	8.09	11.01	2.05	3.56	6.01	8.57	1.59	3.41	4.91	7.53	2.05	2.31	2.45	4.70
Crude fibre(%)	7.69	7.81	7.07	7.41	7.43	7.38	7.49	7.47	5.64	5.61	5.60	6.01	5.07	4.91	4.03	4.01
Ash (%)	8.01	8.03	8.41	8.50	8.09	8.61	8.19	8.08	7.72	7.69	7.71	7.81	7.70	7.57	7.49	7.53
Lysine calculated	5.68	6.54	6.54	6.51	7.83	7.90	9.30	8.41	7.49	7.46	7.43	7.37	8.30	8.65	8.30	6.17
Methionine calculated	2.76	3.40	3.40	3.50	3.77	3.76	5.33	4.02	3.59	3.54	5.52	4.25	3.83	4.20	4.20	3.58

Growth and Nutrient Utilization indices: Weights of fish and feed consumption were obtained at weekly intervals. From the fish weights and feed consumption, the following were determined:

Weight gain = $W_1 - W_0$ (g)

Relative Weight Gain (RWG %) = $(W1 - W0)/W0 \times 100$ (%)

Specific Growth Rate (SGR %) = $\frac{(\ln W1 - \ln W0)}{T} \times 100$ (%/week)

Where;
W_0: mean initial weight (g)
W_1: mean final weight (g)
T: time in 7 days between weightings

Feed conversion ratio (FCR) = $\frac{\text{feed intake (g)}}{\text{wet weight gain}}$ (g)

Protein efficiency ratio (PER) = $\frac{\text{weight gain}}{\text{protein intake}}$

Net protein utilization (NPU) = $\frac{BP1 - BP0}{CP} \times 100$

Where;
BP_0: Initial body protein content (g)
BP_1: Final body protein content (g)
CP: Protein intake (g)

Statistical Analysis: At the end of the experiments, recorded data were subjected to two-way ANOVA test using a Genstat software eight edition, 2005 package for statistical problems. All the means we're compared at 5% level of probability with Duncan multiple range tests. Similarly, responsiveness of fingerlings to treatments was evaluated.

Results

The highest final weight of fish was obtained in the 40% crude protein diet treatment (at DE of 2400), kca/kg and on the 35% crude protein diet at DE levels of 2800 and 3000 kca/kg. the lowest final weight was obtained in a fish fed in the diet containing 25% crude protein all DE levels studied. The differences in final weight between the group fed 35% crude protein and 40% crude protein were not significant at DE levels of 35% crude protein and 2400, 2600, 2800 and 3000 kca/kg diets. In all but one case (at 2400kca/kg), the values of final weights (g) obtained with the 35% crude protein diet were not significantly different (P>0.05) from those obtained in the 30% crude protein diet. Considering the protein level only (ignoring the DE level) the highest final weight was obtained with fish fed with 35% crude protein level although the difference between the 45%, 35% and 30% crude protein dietary treatment were not significant (P>0.05). Considering protein level alone (ignoring the DE level), the total weight gained, the specific weight gain (SGR) and relative weight gain increase as the protein level increase from 25% to 40%. However, the difference between 40% crude protein and 35% crude protein were not significant (P>0.05) except in the case of SGR, FCR and MPU where the differences in the is parameters between 35% and 30% crude protein were significant (P<0.05).
The highest weight gain, daily weight gain, specific growth rate and relative weight gain were obtained in the 40% crude protein diet at energy levels of 2400, 2600 and 2800Kcal/kg and decreased at DE of 3000Kcal/kg at 35% crude protein level. Except for diet containing 2400Kcal DE/kg, the differences in weight gain, SGR and RWG between the groups fed 35% crude protein and those fed 40% crude protein diet had significantly higher weight gain than the 35% crude protein group. Except for the fish fed the diet containing 2400Kcal/kg, the differences in RWG and SGR between the 35% and 30% groups were not significant (P>0.05). Fish fed the 2600Kcal/kg diet containing 35% crude protein was superior to 30% crude protein in terms of weight gain and SGR. Taking the effects of crude protein levels alone while ignoring the DE levels the total weight gain, DWG and RWG increased at the dietary crude protein level increased. However, the differences between 40 and 35%, and 35 and 30% were not significant (P>0.05). Generally, the 25% crude protein dietary treatments had significantly lower weight gain than the other crude protein diet treatments. The 40% crude protein diet gave significantly higher SGR (P<0.05) than the 35% crude protein diets. The differences among RWG and SGR values from dietary protein 35%, 30% and 25% were not significantly different (P>0.05).
No pattern was established on the effects of dietary protein levels on total feed intake and FCR at each energy level. Also, no pattern was established on the effects of digestible energy level on these parameters at each

dietary protein level. However, when the data were summarized on the basis of DE level (ignoring the protein level), feed intake decreased significantly with increase in DE level. No pattern could be established with respect to the effects of dietary protein level (ignoring the energy level) on total feed intake, although the highest feed intake was obtained on the diet containing 35% crude protein and lowest level on that containing 25% crude protein. The FCR decreased with increase in dietary DE with the values obtained in the diets containing 2800 and 3000Kcal/kg diet significantly lower than that obtained with the diet containing 2400Kcal/kg. The Protein efficiency ratio (PER) decreased significantly with increase in dietary protein up to 35% CP and increased at 40% CP. PER increase with increase in DE level and decreased with increase CP level upto 35% increase, thereafter, while the NPU increased significantly with increase in DE level. From the overall results, it can be concluded that the best body weight values (body weight gain, RWg and SGR), were obtained at protein levels of 35 and 40% and energy levels of 2600, 2800 and 3000Kcal/kg were not significantly different (P>0.05). The best FCR were obtained at crude protein levels of 30 and 40% and energy levels of 2600, 2800 and 3000Kcal/kg. it appears that for *C. gariepinus* the optimum protein levels lies between 35 and 40% at the digestible energy value of 2600 or 2800Kcal/kg.

Table 3: Effect of dietary protein and energy levels on growth performance and feed utilization by *Clarias gariepinus* fingerlings

Parameter			**Protein (%)**					**DE Kcal/kg**		
	25	**30**	**35**	**40**	**SEM**	**2400**	**2600**	**2800**	**3000**	**SEM**
Total Weight gain	18.60^{c}	23.0^{b}	25.24^{ab}	27.31^{a}	1.71	25.40^{ab}	25.71^{a}	22.74^{bc}	20.31^{c}	1.36
Relative weight gain	114.6^{b}	116.9^{b}	145.1^{ab}	183.0^{a}	25.20	167.7^{a}	159.0^{a}	126.9^{ab}	106.1^{b}	24.19
Absolute growth rate (g/fish/day)	0.02^{b}	0.02^{b}	0.02^{b}	0.03^{a}	0.01	0.03^{a}	0.02^{b}	0.02^{b}	0.02^{b}	0.01
Specific growth rate (SGR)	0.49^{b}	0.47^{b}	0.53^{b}	0.63^{a}	0.04	0.58^{a}	0.55^{ab}	0.51^{ab}	0.47^{b}	0.05
Feed intake (g)	55.29^{c}	63.09^{b}	76.95^{a}	63.90^{b}	0.96	83.22^{a}	70.59^{b}	59.97^{c}	45.45^{d}	0.98
Feed conversion ratio (FCR)	2.97^{b}	2.74^{ab}	3.05^{b}	2.34^{a}	0.23	3.28^{c}	2.75^{bc}	2.64^{b}	2.34^{a}	0.21
Crude protein intake (CP) (g)	13.82^{d}	18.93^{c}	26.93^{a}	25.56^{b}	0.04	26.78^{a}	23.17^{b}	20.17^{c}	15.15^{d}	0.03
Protein efficiency ratio (PER)	1.35^{a}	1.22^{b}	0.94^{c}	1.07^{c}	0.04	0.95^{d}	1.11^{c}	1.13^{b}	1.34^{a}	0.06
Net protein utilization (NPU) (%)	28.99^{a}	21.71^{b}	16.27^{d}	18.33^{c}	0.26	17.10^{d}	20.17^{c}	22.48^{b}	25.55^{a}	0.22

NB: Within protein or energy levels, values in a column with similar superscripts are not significantly different (P>0.05)

Table 4: Effect of varying dietary levels of protein and energy on the growth performance and feed utilization by *Clarias gariepinus* fingerlings.

Dietary Treatment	TWG	RGR	AGR	RWG	SGR	FI	FCR	PI	PER	NPU
A 2400Kcal/kg										
25% protein	27.87^{abc}	0.01^{cd}	0.06^{ef}	111.57^{cde}	0.47^{bcde}	97.2^{a}	5.44^{e}	24.30^{a}	0.74^{d}	7.24^{h}
30% protein	27.88^{abc}	0.03^{abc}	0.10^{abc}	211.10^{abc}	0.66^{abc}	65.61^{ef}	2.35^{ab}	19.68^{b}	1.42^{b}	12.55^{d}
35% protein	34.24^{a}	0.02^{abcd}	0.09^{bcd}	118.90^{bcde}	0.46^{bcde}	98.1^{a}	4.01^{d}	34.34^{a}	0.71^{d}	11.01^{i}
40%protein	31.59^{ab}	0.04^{a}	0.11^{a}	241.20^{a}	0.74^{a}	72.00^{d}	2.28^{ab}	28.80^{a}	1.10^{c}	10.07^{f}
B 2600Kcal/kg										
25% protein	20.19^{cd}	0.02^{abcd}	0.07^{def}	137.30^{abcde}	0.47^{bcde}	59.40^{g}	2.94^{c}	14.85^{c}	1.36^{b}	7.07^{c}
30% protein	33.29^{ab}	0.01^{d}	0.08^{cde}	213.12^{ab}	0.40^{e}	78.99^{b}	3.39^{c}	23.70^{a}	0.98^{d}	12.95^{g}
35% protein	29.83^{ab}	0.03^{ab}	0.11^{ab}	224.00^{ab}	0.68^{a}	69.30^{de}	2.32^{ab}	24.26^{a}	1.23^{b}	9.07^{e}
40%protein	29.54^{abc}	0.03^{abcd}	0.11^{ab}	183.70^{abcd}	0.64^{cde}	74.7^{c}	2.53^{abc}	29.88^{a}	0.99^{d}	7.73^{g}
C 2800Kcal/kg										
25% protein	18.60^{d}	0.02^{abcd}	0.07^{def}	148.30^{abcde}	0.56^{abcd}	33.99^{i}	1.83^{a}	8.50^{d}	2.19^{a}	12.59^{b}
30% protein	30.72^{ab}	0.01^{d}	0.07^{def}	66.30^{e}	0.40^{e}	61.89^{fg}	2.99^{bc}	18.57^{b}	1.12^{c}	16.10^{f}
35% protein	33.59^{a}	0.02^{abcd}	0.09^{bcd}	108.00^{cde}	0.45^{cde}	81.00^{b}	3.43^{c}	28.35^{a}	0.83^{d}	17.22^{h}
40%protein	28.06^{abc}	0.03^{abcd}	0.10^{abc}	184.80^{abcd}	0.65^{abcd}	63.00^{fg}	2.25^{ab}	25.20^{a}	1.11^{c}	8.73^{f}
D 3000Kcal/kg										
25% protein	17.73^{d}	0.01^{d}	0.06^{t}	73.20^{d}	0.44^{cde}	30.60^{i}	1.73^{a}	7.65d	2.32^{a}	3.53^{a}
30% protein	20.16^{d}	0.01^{cd}	0.07^{def}	99.30^{cde}	0.43^{de}	45.90^{h}	2.28^{ab}	13.77^{c}	1.46^{b}	1.16^{c}
35% protein	23.30^{c}	0.02^{abcd}	0.08^{cde}	129.50^{abcde}	0.51^{bcde}	59.40^{g}	2.55^{abc}	20.79^{b}	1.12^{c}	4.57^{f}
40%protein	20.06^{d}	0.02^{bcd}	0.07^{def}	122.30^{bcde}	0.49^{bcde}	45.90^{h}	2.29^{ab}	18.36^{b}	1.09^{c}	3.65^{f}
SEM	2.723	0.01	0.001	48.37	0.09	1.96	0.42	0.60	0.12	0.43

NB: Within protein or energy levels, values in a column with similar superscripts are not significantly different ($P>0.05$)

TWG - Total Weight gain
AGR - Absolute growth rate (g/fish/day)
RWG - Relative weight gain
SGR - Specific growth rate (SGR)
RGR - Relative growth rate (RGR) (g/day)
FI - Feed intake (g)
FCR - Feed conversion ratio (FCR)
CP - Crude protein intake (CP) (g)
PER - Protein efficiency ratio (PER)
NPU - Net protein utilization (NPU) (%)

Discussion

The highest weight gain, absolute growth rate (AGR) and specitic grow rate SGR) in *C gariepinus* were obtained at a crude protein (CP) level of 35 a digestible energy level DE level of 2600kcal/kg diet) and 40% CP (at DE level of 2400kcal/kg diet). However, the differences between these two diets in terms weight gain, AGR and SGR were not significant.

Therefore, dietary crude protein level of 35% could be recommended as the optimum level. Feed intake increased with increase in dietary crude protein level up to 35% and decreased thereafter. Similar responses were reported by Sabut and Luquet (1973) for the golden beam, Chrysophyrys auratus, Cowey et al. (1972) for carp, Cyprinus carpio, Jauncey (1982) for the cichlid Oreochromis mossambicus, De Silva et al. (1989) for

Oreochromis niloticus, Martinez-Palacios et al (1996) for Cichlasoma uropthalmus and Obasa and Faturoti (2004) for Chrysichthys walkeri. This value fell within the acceptable concentration ranges for the channel catfish Boyd (1979). In all these studies, specific growth rate SGR these two diets in terms of peaked at the optimum crude protein level and decreased thereafter. This observation can be attributed to the fact that as the dietary CP 1increases, the quality of feed improves and this encourages enhanced feed intake. The increasing feed intake as the protein level in the diet increased could also be attributed to the acceptability effect of high protein levels in the diet, since protein quality and quantity are among the factors regulating feed intake in animals (Pond, 1965),

On the basis of Feed conversion ratio (FCR), the difference between the 35% and 40% CP diet, at all DE levels tested were not significant (P>0.05). The recommended crude protein (CP) level of 35% is in agreement with a CP recommended for *H. longifilis* fingerlings (Eyo and Falayi, 1999), 35% CP Tor the catfish *Chrysicthys nigrodigitatus* (Erondu et al, 2000) 35-40% for *C. gariepinus* (ADCP, 1983) and 35% for all catfish (Olomu, 2011) but at variance with 40% CP recommended for *Clarias gariepinus* (Faturoti el al., 1980); 40% for *Clarias anguillaris* (Madu, 1992); 40-42% for *H. bidorsalis* (Eyo 1990; Fagbenro et al, 1992); and 42.5% for *H. longlfilis* (Eyo et al., 1998), The slightly higher CP levels of 40 to 42.5% recommended by some researchers for pure bred fingerlings may be due to a number of reasons.

The results from the present study showed that feed conversion ratios (FCR) were optimized at different crude protein level under different energy level is a reflection of the relationship existing between the protein and energy requirement of fish. This observation emphasizes the fact that in the formulation of feed for fish, protein should not be considered in isolation of the dietary energy level; hence the importance or the optimum calorie: protein ratio recommended. In addition, an important observation is that the amount of energy consumed decreased significantly as the diet DE increased. Thus the results did not support the assertion that fish fed a wide range of dietary energy levels, adjust feed intake in order to provide fixed energy consumption, under ad libitum feeding regimes. In the present studies, the experimental fish were fed to satiation and not ad libitum.

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