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Optimum Dietary Crude Protein and Digestible Energy Requirements for fingerlings of Hybrid Clariid catfish *Clarias gariepinus*♀ *X Heterobranchus bidorsalis*♂ in the Tropics

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ABSTRACT

This study was carried out to determine the optimum dietary crude protein and digestible energy requirements for the cross *Clarias gariepinus*♀ *X Heterobranchus bidorsalis*♂ fingerlings. The general objective of the study was to determine the optimum protein and digestible energy levels for the Clariid catfish fingerlings, cross *Clarias gariepinus*♀ *X Heterobranchus bidorsalis*♂, using locally sourced feed inputs. *Clarias gariepinus*♀ *X Heterobranchus bidorsalis*♂ fingerlings were reared from hatchlings to five weeks old. Sixteen practical diets were formulated and used in the feeding trials. The diets were made up of four digestible energy levels (2400, 2600, 2800 and 3000Kcal/Kg), each at four crude proteins levels (25, 30, 35 and 40%) and were fed to the fingerlings for 70days in three replicates for each treatment. Weekly data were collected based on weight gain and feed consumption. Feed and fish carcasses were analyzed for proximate composition of the fingerlings. All data collected were subjected to two-way analysis of variance (ANOVA) test at 5% probability level. The result showed that weight gain increased with increase in the dietary protein levels at all energy levels used. The increases were significant in all except that between 35% crude protein and 40% crude protein at DE of 2600Kcal/kg diet. The highest weight gain was obtained in fish fed diet containing 40% crude protein and DE 2600Kcal/kg diet. These values obtained were not significantly different (P>0.05) from those obtained in fish fed diet containing 35% crude protein and DE of 2600Kcal/kg. Feed intake at DE levels of 2400 and 2600 Kcal/kg diets were similar and significantly higher (P<0.05) than feed intake at 2800 and 3000Kcal/kg. Generally, beyond 2600Kcal/kg diets, feed intake reduced significantly. Specifically, feed intake reduced when the DE level was increased from 2400 to 2600Kcal/kg at crude protein levels of 25 and 30% but increased at crude protein levels of 35 and 40%.The FCR values were not significantly affected by treatments (P>0.05). However, it is only the FCR obtained in the diets containing 25% crude protein and DE of 2400kcal/kg that was significantly different from all the others. The lowest FCR (0.84) was obtained in fish fed diet containing 25% crude protein and DE of 3000kcal/kg followed by that (1.14) of 35% crude protein and DE of 2600kcal/kg diet. At each protein level, the FCR decreased with increase in DE levels, although not significantly. At DE of 2400, 2600 and 2800kcal/kg, the FCR reduced with increase in the crude protein level up to 35%.

Keywords: Clariid catfish, *Clarias gariepinus*, fingerlings, optimum energy, protein levels, fish diet.

INTRODUCTION

Fish is a key ingredient on the global menu, vital factor in the global environmental balance, and an important basis for livelihood worldwide (UNICEF, 2006). Fish has no cultural or religious restrictions which makes it more advantageous than pork, beef and mutton (NIFFR, 1999) Fish is an indispensable source of micronutrients, such as iron, iodine, zinc, vitamin A and B (World Fish

Centre, 2005). Present knowledge of the chemical proximate composition of fish species from Nigerian waters is scanty. The measurement of some nitrogen free extract and crude fiber is often necessary to ensure that they meet the dietary requirements and commercial specification (Onyia *et al*., 2010).

Nutritionally, fish is about the cheapest and direct source of protein and micro nutrients for several millions of Africans (Bene and Heck, 2005). It provides 40% of animal protein consumption in Nigeria and it is also a very important source of animal protein for livestock in developed and developing countries (Ozigbo et al., 2014). In Nigeria, fish demand as estimated by Ruma, (2008) was 2.1 million metric tons at 11.5kg per capita consumption with domestic production from the wild estimated at 5% leaving a gap of 41% which is about four times the level of local production.

It should be noted that knowledge of the protein requirement of fish is essential for the formulation of a well-balanced artificial diet for economical fish feeding (Omoniyi and Fagade, 2003). Protein requirement is linked with the general energy requirement of the fish at a given water condition and the ability of the fish to gain weight at its inherent capacity (Eyo, 20003), protein and energy levels significantly influence food conversion effect of *Heterobranchus bidorsalis* however; the efficiency was not high enough to influence carcass composition and condition of fish. According to Falaye (1992), the nutrient requirements of fish depend on the age, species, production function and environmental condition.

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♀ X Heterobranchus bidorsalis♂* 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: 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.

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

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%)=$(W_1 – W_0) / W_0 \times 100$ (%)

Specific Growth Rate (SGR %)= $\{(\text{In } W_1 – \text{In } W_0)/ T\} \times 100$ (%/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) = feed intake (g) / wet weight gain (g)

Protein efficiency ratio (PER) = weight gain (g) / protein intake (g)

Net protein utilization (NPU) = $\{(BP_1 - BP_0)/ CP\} \times 100$
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.

RESULT

The result showed that weight gain increased with increase in the dietary protein levels at all energy levels used. The increases were significant in all except that between 35% crude protein and 40% crude protein at DE of 2600Kcal/kg diet. The highest weight gain was obtained in fish fed diet containing 40% crude protein and DE 2600Kcal/kg diet. These values obtained were not significantly different (P>0.05) from those obtained in fish fed diet containing 35% crude protein and DE of 2600Kcal/kg. The lower protein levels resulted in very low weight gain. At each protein level, DE did not have significant effect on the weight gain. The effects of dietary treatments on SGR, RGR and RWG were similar to those described for the weight gain.

At each DE level, the difference in feed intake by fish fed diets containing 25 and 30% crude protein were not significantly different (P>0.05). Feed intake on these two protein levels was very low. At dietary protein levels of 35% and 40% the amount of feed consumed were significantly higher (P<0.05) than those recorded at dietary protein levels of 25% a 30%. Except for 35 and 40% crude protein at DE of 2600, the amount of feed consumed by the fish fed the 35% crude protein diets were significantly lower(P<0.05) than those of fish fed the diets containing 40% crude protein. Feed intake at DE levels of 2400 and 2600 Kcal/kg diets were similar and significantly higher (P<0.05) than feed intake at 2800 and 3000Kcal/kg. Generally, beyond 2600Kcal/kg diets, feed intake reduced significantly. Specifically, feed intake reduced when the DE level was increased from 2400 to 2600Kcal/kg at crude protein levels of 25 and 30% but increased at crude protein levels of 35 and 40%.

The FCR values were not significantly affected by treatments (P>0.05). However, it is only the FCR obtained in the diets containing 25% crude protein and DE of 2400kcal/kg that was significantly different from all the others. The lowest FCR (0.84) was obtained in fish fed diet containing 25% crude protein and DE of 3000kcal/kg followed by that (1.14) of 35% crude protein and DE of 2600kcal/kg diet. At each protein level, the FCR decreased with increase in DE levels, although not significantly. At DE of 2400, 2600 and 2800kcal/kg, the FCR reduced with increase in the crude protein level up to 35%. When the FCR values were the basis of protein levels ignoring the DE levels, differences in DE levels and FCR values obtained, were not significantly different (P>0.05). However, FCR decreased with increase in dietary crude protein level up to 35% and decreased linearly with increase in dietary energy level.

Protein efficiency ratio (PER) decreased with increase in protein level but increased with increase in digestible energy (DE) level. Both caloric and protein intake increased with increase in DE and protein levels respectively. The differences in dietary crude protein or energy levels did not significantly affect the carcass composition of fish moisture, crude protein, fat and ash. The initial and final compositions were not significantly different.

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

Parameter	Protein (%) DE Kcal/kg									
	25	30	35	40	SEM	2400	2600	2800	3000	SEM
Total Weight gain	21.50^{b}	22.00^{b}	107.50^{a}	136.80^{a}	21.50	72.60^{NS}	85.50^{NS}	65.90^{NS}	63.70^{NS}	21.60
Relative weight gain	340^{b}	432^{b}	142^{a}	187.30^{a}	40.00	1136^{NS}	1091^{NS}	885^{NS}	945^{NS}	33.90
Absolute growth rate (g/fish/day)	0.078^{b}	0.079^{b}	0.384^{a}	0.47^{a}	0.10	0.258^{NS}	0.31^{NS}	0.236^{NS}	0.215^{NS}	0.15
Specific growth rate (SGR)	0.909^{b}	0.941^{b}	1.077^{a}	1.73^{a}	0.15	1.454^{a}	1.352^{ab}	1.176^{b}	1.28^{ab}	0.12
Feed intake (g)	34.95^{c}	34.65^{c}	150.39^{b}	202.29^{a}	2.61	133.05^{a}	133.65^{a}	88.97^{ab}	66.75^{c}	2.66
Feed conversion ratio (FCR)	1.63^{NS}	1.58^{NS}	1.40^{NS}	1.48^{NS}	0.08	1.83^{NS}	1.56^{NS}	1.35^{NS}	1.05^{NS}	0.08
Crude protein intake (CP) (g)	8.74^{c}	10.40^{c}	52.64^{b}	80.92^{a}	2.02	47.31^{b}	45.36^{b}	21.78^{c}	24.48^{c}	2.13
Protein efficiency ratio (PER)	2.46^{a}	2.12^{ab}	2.04^{b}	1.69^{b}	1.01	1.54^{c}	1.89^{b}	3.03^{a}	2.60^{a}	1.03
Net protein utilization (NPU) (%)	44.11^{a}	34.29^{b}	33.39^{b}	25.07^{c}	1.01	24.36^{d}	29.45^{c}	36.76^{b}	40.07^{a}	1.03

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♀ X Heterobranchus bidorsalis♂* fingerlings.

Dietary Treatment		TWG	RGR	AGR	RWG	SGR	FI	FCR	PI	PER	NPU
A	2400Kcal/kg										
	25% protein	24.70e	0.06b	0.09b	411.67b	1.02bcd	57.60a	2.27a	14.40f	1.72b	19.24fg
	30% protein	31.50e	0.13ab	0.11b	984.38ab	1.37ab	59.40a	1.89a	17.82e	1.77b	23.74fg
	35% protein	101.10de	0.21ab	0.29ab	1366.22ab	1.67a	182.40c	1.80a	63.84c	1.58b	22.20fg
	40%protein	132.90	0.25ab	0.42ab	1620.73ab	1.76a	232.80a	1.75a	93.12a	1.43b	19.57g
B	2600Kcal/kg										
	25% protein	20.60e	0.04b	0.07b	282.19b	0.83d	38.10fg	1.85a	9.53efg	2.16b	22.67e
	30% protein	24.10e	0.05b	0.09b	301.43b	0.93cd	37.50fg	1.56a	11.25efg	2.14b	23.02e
	35% protein	145.20a	0.20ab	0.53a	2016.67ab	1.85a	219.60ab	1.51a	76.86b	1.89b	24.82ef
	40%protein	152.00a	0.33a	0.62a	2576.27a	1.79a	239.40a	1.58ab	95.76a	1.59b	23.27fg
C	2800Kcal/kg										
	25% protein	21.10e	0.04b	0.08b	246.00b	0.80d	27.90a	1.32a	6.98fg	3.03b	28.37b
	30% protein	17.70e	0.04b	0.07b	242.47b	0.76d	24.00g	1.36a	7.20fg	2.46b	21.81d
	35% protein	92.00de	0.18ab	0.37ab	1164.80ab	1.51ab	105.00e	1.14b	36.75d	2.50b	34.83cd
	40%protein	133.00a	0.26ab	0.48a	1683.54ab	1.63a	198.30bc	1.49ab	79.32b	1.6ab	24.12fg
D	3000Kcal/kg										
	25% protein	19.40e	0.06b	0.07b	280.39b	0.98cd	26.20g	1.35a	6.55g	2.96a	33.28a
	30% protein	14.50e	0.03b	0.06b	198.63b	0.71d	27.70g	1.91a	8.31fg	1.75b	12.88cd
	35% protein	91.60de	0.22ab	0.30ab	1327.54ab	1.67a	94.50e	1.03a	33.08d	2.77b	38.69bc
	40%protein	129.31c	0.24ab	0.27ab	1724.13ab	1.75a	138.60d	1.07a	55.44c	2.33b	33.66ef
	SEM	4.32	0.10	0.02	6.618	0.236	10.65	0.33	1.42	0.69	2.07

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, AGR and second best SGR were obtained with 40% CP diet and 2600kcal/kg diets. On this basis, 40% crude protein level could be recommended as optimum for fingerlings of this reciprocal cross. With FCR as criteria, the differences between diets containing 35% and 40% crude protein were significant (P<0.05). Thus a CP level, 35% could be recommended. With FCR as criteria for judgment thus suggesting that based on the criteria for judgment, different optimum CP could, be recommended.

Indications for higher proteins requirements for hybrid in this study substantiate earlier reports which showed that hybrids require higher dietary crude protein level than the pure breeds (Eyo, 1995, Eyo and Olatunde, 2002). The need for a higher protein requirement for hybrids compared to pure breeds may be explained on the basis that the hybrid attained higher body weights than the pure breeds. Thus the higher protein requirement might be related to the need for protein to support the higher genetic potential of the hybrid to grow faster than the pure breeds.

The feeding regimen may also contribute to differences in optimum crude protein levels recommended, Ali (2001) reported from the study with C gariepinus that the recommended CP level for fingerlings could be reduced from over 40% to 35% by feeding to satiation instead of at fixed rate, at an optimized calorie/protein ratio, Martinez Palacios et al. (1996) reported that when Mexican cichlid fish (Ciehlasomaurophthalmus) were fed a fixed rate diet, the optimum CP level ranged between 43.5 and 56% but when fed to satiation, the optimum CP level was 32.5, This reduction occurred because when the feeding rate of fishes increases, the protein requirement is reduced because the fish are able to compensate for the reduced dietary CP by consuming more diet (Cowey and Tacon, 1985). However, the recommended CP level of 359% reported from the present investigation is above that (32%) recommended for channel catfish by NRC (1995). The requirement by NRC (1995) was determined with highly purified ingredients in which the nutrients were highly digestible and therefore the value presented represent near 100% availability. The 35% CP determined in this study is based on practical feed ingredients with possibly lower availability. Another reason may be attributed to differences in age of the fish. This reason is supported by the recommendation of over 40%, 35% and 30% respectively for *C. gariepinus* fry. juvenile and adult/broodstock showing that recommended crude protein level vary with age and size of fish, adult/blood stock (Faturoti, 2003).

Also, the total teed intake increased with increase in CP level up to 40%. This observation is Similar to that made with the pure breed when feed intake increased up to the optimum protein level (55%) and decreased thereafter. This observation seems to indicate that the optimum level for the reciprocal crosses could be higher than 40% since feed intake was increasing. Further studies are required to elucidate this point. The energy levels, (2400 or 26600kcalkg diet) recommended is less than that (2800 to 3140kca/kg diet) recommended for channel catfish in the temperate zone (Xiangha, 1986. Li and Lovel, 1992, Mangalik, 1986; Garling and Wilson 1978) and 3429kcalkg for catfish (Smith, 1980) and that (3000kcal/kg diet) assumed by NRC (1995) as the typical DE of commercial diets. Other recommendations which are much higher than the present recommendation include, 3050kcal/kg ME and Faturoti (2003) who recommended energy level of 3200 and 3400kcal/kg ME respectively for *Clarias gariepinus* and *Heterobranchus bidorsalis*, Obasa and Faturou (2004) who reported better performance on 3000kcal/MEkg diet than on 2800kcal/kg for brackish water catfish (*Chrysichthys walker*).

A major reason why the results of energy requirements obtained in this study cannot be compared with other energy requirements recommended elsewhere in Nigeria is due to the differences in energy used. While some researchers used metabolizable energy as their measure, (Obasa and Faturoti, 2004), Other workers such as Dada *et al.* (2001) and Ovie*et al.* (2005) used gross energy in their studies. The present requirements were based on digestible energy. It is therefore proposed that a standard measure of energy be adopted for energy studies in fish. Gross energy is not a practical measure or indicator of usable energy because certain ingredients are not as digestible as others. Moreover, some ingredients may have high gross energy value but have low or no digestible energy value. Metabolizable energy would appear to be slightly superior to digestible energy since it is a more precise measure of available energy. However, it is more cumbersome to determine the ME of feed ingredients for fish. There is also little evidence to show that the extra work needed in determining ME makes ME more valuable than DE as an energy measure for fish. Digestible energy values are easier to determine and the fish are not stressed when allowed to feed voluntarily (Page and Andrews, 1973, Takeuchi et al., 1980). In practice, ME offers little advantage over DE because energy loss in digestion account for most of the variation in losses or gross energy (NRC, 1995). There also appears to be a high correlation between DE and ME (Devendra, 1989).

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