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CONTRIBUTION TO THE STUDY OF THE EFFECTS OF VARIOUS ADDITIVES OF ANIMAL ORIGIN ON THE GROWTH OF HYBRID CLARIAS FRY (MALE *CLARIAS GARIEPINUS* X FEMALE *CLARIAS ANGUILLARIS)*

Abstract

Aquaculture in Senegal faces constraints such as the high cost of fish feed, representing a brake on the sector's development. In this study, alternative local meals (cow blood meal, chicken viscera meal and black soldier fly larvae meal) were used as feed additives for hybrid fry of *Clarias anguillaris* and *Clarias gariepinus*. Four experimental diets (R1, R2, R3 and R4) were formulated, in addition to an industrial control diet (R0), to assess their effects on growth, feed efficiency and production cost. 300 fry of initial mean weight of 1.56 g were distributed in 15 tanks of 50 liters. After 45 days of experimentation, the best zootechnical performance was obtained with diet R4, containing the combination of the three additives. This diet showed a relative mean weight gain (RMWG) of 521.58% and a feed conversion ratio (FCR) of 1.44, similar to the control diet. Economically, the R4 diet had the lowest feed cost of 666.41 FCFA/kg and fish cost of 958.94 FCFA/kg, while maintaining a high survival rate (95%). These results demonstrate that the use of local ingredients as additives is a sustainable and cost-effective alternative for improving the productivity and competitiveness of aquaculture in Senegal.

Keywords: Cow blood meal, chicken viscera, black soldier fly larvae, growth, feed efficiency, cost

Introduction

Fishery plays a considerable socio-economic role in Senegal. However, given the difficulties associated with effective fisheries management and the growing demand for fisheries resources, Senegal has been faced for several decades with the effects of overfishing of its ecosystems, climate change and the collapse of fisheries, impacting local consumption of fisheries production, food security and economic growth (Baldé, 2019). Faced with this situation, the Senegalese government has been committed since 2007 to promote aquaculture as an alternative way of coping with dwindling fisheries resources (Ndiaye, 2019).

Aquaculture plays a crucial role in food security and economic development, particularly in developing countries. However, one of the major obstacles to its expansion remains the high cost of fish feed, representing between 40% and 60% of total production costs (Siddhuraju and Becker, 2003). This dependence on imported feed limits farm profitability and hinders the development of high-potential species such as *Clarias gariepinus* and *Clarias anguillaris*, renowned for their rapid growth and adaptability. In this context, the use of local by-products to formulate less costly diets represents a sustainable and economically viable alternative.

In Senegal, products such as chicken viscera, cow blood and black soldier fly larvae are often under-exploited. Slaughterhouses discharge their effluents, including blood, into drainage channels without pre-treatment, contributing to significant environmental pollution. These by-products, which are rich in essential nutrients, nonetheless offer potential for valorization. Blood, for example, contains high-quality proteins, notably albumin and hemoglobin, and its recovery reduces the biochemical oxygen demand (BOD) of wastewater, thus limiting the degradation of aquatic ecosystems (WHO, 2023). Chicken viscera, also rich in proteins, fats and amino acids, are often regarded as waste, although they can be processed into meal for animal feed. Black soldier fly larvae, on the other hand, are rich in protein and essential fatty acids, and have the advantage of being produced from recycled organic matter, making them an ecological and sustainable source.

The general aim of this study was to evaluate the effects of these ingredients, used as feed additives, on the growth of catfish hybrid fry of male *Clarias gariepinus* and female *Clarias anguillaris*. More specifically, it aims to:

- add value to cow blood, broiler viscera and black soldier fly larvae by incorporating them as additives in Clarias hybrid feeds;
- determine the zootechnical performance of Clarias hybrids;

- determine the nutritional contribution of foodstuffs on the flesh of Clarias hybrids;
- determine variations in rearing water temperature and pH during experimentation;
- determine the production cost of feed and fish.

Material and methods

Methods

50 **Processing of some ingredients used in this study**

Various methods were used in this study. These included processing of raw materials into finished products, feed formulation and manufacture, rearing conditions and determination of zootechnical parameters of *Clarias* hybrid fry.

Blood meal production

48 Fresh blood obtained from the SOGAS slaughter house in Dakar was placed in a 20L plastic container and transported to the IUPA aquaculture station. To sterilize the fresh blood, it was heat-treated by boiling at 120 degrees Celsius for a quarter of an hour, then sun-dried for 48 hours and finely ground for use in diets. The blood meal was stored in glass jars until use.

30 **Black soldier fly meal production**

Black soldier fly larvae were harvested when they reached an optimal stage of development, generally after 7 to 14 days of rearing.

After harvesting, the larvae were immobilized with hot water. Once treated, they were spread out in the sun to dry and passed through a press to extract the oil. Finally, the dried and defatted larvae were ground to obtain a fine flour.

Chicken viscera meal production

Chicken guts obtained fresh from the market were meticulously washed and rinsed. This step was crucial to remove impurities, food residues and contaminants, thus ensuring the cleanliness of the intestines prior to processing. After this stage, they were then subjected to heat treatment by boiling at 100 degrees Celsius for a quarter of an hour. After draining, the viscera were spread out in the sun to dry. Once dried, they were ground into a fine flour and preserved in glass jars.

Diets formulation

Four experimental diets were formulated using the Pearson square method, and an industrial feed was used as a control. Table I provides information on the centesimal composition of the different ingredients used and the composition of the different ingredients in the industrial feed, as well as their bromatological compositions.

Table I: Centesimal composition of the four formulated diets

Ingredients	R1 (viscera)	R2 (viscera + blood)	R3 (viscera +BSF)	R4 (viscera + blood +BSF)	Industrial food
Fish meal	35	35	35	35	Fish meal,
Soybean meal	10	10	10	10	Soya cake meal, Wheat meal,
Peanut meal	10	10	10	10	Soya oil,
Wheat bran meal	10	10	10	10	Maize meal,
Corn flour	10	10	10	10	
Cassava flour	9	9	9	9	Rice bran,

Chicken viscera meal	5	2,5	2,5	1,66	Distillers Dried Grains with Solubles (DDGS) and Maize gluten
Cow's blood meal	0	2,5	0	1,67	
BSF larvae meal	0	0	2,5	1,67	
Fish oil	5	5	5	5	
Premix vitamins[a]	1	1	1	1	
Premix minerals[b]	1	1	1	1	
Yeast	4	4	4	4	
Total	100	100	100	100	
Crude Protein (%)	37,5	37,5	37,5	37,5	37
Crude lipid (%)	11,8	11,37	11,43	11,44	8
Cost of feed (F CFA)	657,22	654,72	691,43	666,415	1050

a=vit A 250000 UI; vit D3 250000UI; vit E 5000mg; vit B1 100mg; vit B2 400mg; vit B3(pp) 1000mg; vit B5 pantode Ca2000mg; vit B6 300mg; vit K3 1000g; vit C 5000mg; H biotin 15mg; choline 100g; anti-oxydant (BHT), crushed and calcined attapulgite qs 1000mg; b=phosphorus 7%; calcium 17%; sodium 1,5%; potassium 4,6%; magnesium 7,5%; manganese 738mg; zinc 3000mg; iron 4000mg; copper 750mg; iodine 5mg; cobalt 208mg; calcined and ground attapulgite qs 1000g; fluorine 1.5% (approximately),

Diets manufacturing

The different proportions of ingredients shown in Table III are formulated to produce 1000g of feed. The raw materials for each feed were sieved, weighed and mixed to homogenize them. Water was then added at a rate of 30% of dry matter. This resulted in a malleable dough mixture. This mixture was passed into a Moulinex mincer, from which it emerged in the form of spaghetti filaments. These filaments then underwent fragmentation, followed by sun-drying for two days. Once dried, they were placed in a mortar, then crushed and sieved to obtain flour, which was then preserved in bags.

Breeding conditions

The hybrids used in this experiment were derived from the artificial reproduction of a male *Clarias gariepinus* and a female *Clarias anguillaris*. Three days after hatching, the larvae were fed artemia SEP for 15 days. During the course of the experiment, 300 fry with an initial average weight of 1.56 g were randomly distributed in 15 aquariums of 50-liter, i.e. 20 fry per tank. These fish were acclimatized for one week.

The experiment lasted 45 days. The fish received a daily ration corresponding to 8% of their live weight. This ration was distributed in the morning at 8 a.m. and in the evening at 5 p.m., after temperature and pH measurements had been taken.

Finally, to maintain water quality, the bottom of the rearing tanks was siphoned off each morning before feeding. The edges were cleaned and rinsed thoroughly to limit the deposit of any fatty matter left by the feed.

Evaluation of survival growth parameters, feed efficiency and feed costs

Growth checks were carried out early in the morning after every two weeks of the experiment. Using a landing net, the fish were transferred to a plastic pot placed on a scale. After weighing, the fry were returned to the carefully cleaned aquariums. The results obtained were then entered into an Excel spreadsheet to determine zootechnical parameters for assessing fish growth and feed utilization efficiency. The various zootechnical parameters used as indicators in this experiment are:

Specific Growth rate (SGR, %/d)= (ln(final average weight)-ln(initial average weight)/experiment duration X100 .

Survival Rate (SR, %)= number of final fish / number of initial fish X100 .

Feed Conversion Ratio (FCR) = Feed intake / weight gain .

Absolute Mean Weight Gain (AMWG, g/fish) = average final weight - average initial weight

Relative Mean Weight Gain (RMWG, %) = 100 x ((final average weight - initial average weight)/initial average weight))

Cost of feed (FCFA) = Total costs (raw materials, processing, transport) / Quantity of feed produced per kg

Production cost per kg of fish (FCFA**)**= FCR× Feed cost (per kg)

Data analysis

Analysis of statistical differences between groups was carried out using ANOVA and Duncan test, implemented via SAS software, which is a widely used computer program for statistical data analysis and database management. Created in the 1970s by SAS Institute, it is particularly popular in fields as diverse as social sciences, bio statistics. It enabled us to identify homogeneous groups based on their significant differences. Thanks to SAS' advanced features, the averages were automatically grouped into distinct classes, facilitating interpretation of the results. This method proved effective in highlighting significant differences while controlling the risk of Type I error, in line with the study's objectives

Results and discussion

Results

Yield of main ingredients used

The yields of the main ingredients used as additives are shown in Table II

TableII : Yield of main ingredients used as additives

Ingredients	Initial quantity	Final quantity (g)	Yield (%)
Cow's blood	17 L	7000	41,48
Black soldier fly larvae	1.70 kg	465	27,35

Chicken viscera	20 kg	800	4

Table II shows a significant variation in yields between processed ingredients. Cow's blood shows the highest yield (41.18%), reflecting efficient processing with moderate losses, making it an economically advantageous option. Black soldier fly larvae, with a yield of 27.35%, show losses linked to their high water content, but remain attractive due to their high nutritional value and ecological impact. Chicken intestines, on the other hand, had a very low yield (4%), indicating significant losses during the process, probably due to a high proportion of unusable material. These results suggest that cow's blood and fly larvae should be preferred, while chicken intestine processing needs to be optimized or alternative uses considered to reduce losses and improve recovery

Biochemical composition of main ingredients

6 The various ingredients used, in particular cow blood meal, chicken intestine meal and black soldier fly larvae meal, were sent to LANAC for analysis using the AOAC, 1995

35 method. The results of the biochemical composition are presented in Table III.

Table III: Biochemical composition of blood meal from Black Soldier Fly (BSF) larvae and chicken viscera meal

Biochemical composition	Blood meal	BSF meal	Chicken viscera meal
Fat content (%)	1,97	14,32	19,33
Protein (%)	93,81	32,37	61,25

Water quality parameters (Temperature and pH variations)

TableIV : Changes in temperature and pH during experiment

Parameters	R0	R1	R2	R3	R4
Temperature	28,61±0,005^{a}	28,42±00^{c}	28,45±0,01^{c}	28,45±0,03^{c}	28,50±0,04^{b}

pH	7,83±0,005[a]	8,04±0,02[b]	7,99±0,01[c]	7,96±0,02[d]	7,93±0,01[e]

Table IV shows the results of statistical analyses of water parameters, namely temperature and pH, for five different feeding diets (R0, R1, R2, R3, R4). Values shown include means and their standard deviations, while letters (a, b, c, d, e) show significant differences between treatments.

For temperature, diet R0 (28.61) had a significantly higher temperature than all other diets. Diets R1, R2, and R3 (28.42; 28.45; 28.45°C) had statistically similar temperatures. Diet R4 (28.50°C) was slightly higher than R1, R2, and R3, but still significantly different from R0 and the others.

In terms of pH, diet R0 (7.83) had the lowest pH and was significantly different from the other diets. Diet R1 (8.04) showed the highest pH and was significantly different from all other treatments. Diets R2 (7.99) and R3 (7.96) had close but statistically distinct values. Diet R4 (7.93) has the lowest pH after R0, but remains significantly different from the others.

Zootechnical parameters

During the experiment, various zootechnical parameters were studied to determine fish growth in each diet. These results are shown in Table V.

TableV: Growth, feed efficiency and survival parameters of catfish hybrids.

Parameters	R0	R1	R2	R3	R4
IMW g	1,56[a]	1,56[a]	1,56[a]	1,56[a]	1,56[a]
FMW g	10,62±0,25[a]	5,65±0,15[d]	7,85±0,23[c]	5,82±0,38[d]	9,69±0,11[a]

AMWG g	9,07±0,25^{a}	6,09±3,34^{ba}	6,09±0,49^{ba}	4,27±0,39^{b}	8,13±0,11^{ba}
RMWG%	582,76±16,81^{a}	262,46±17,59^{d}	390,73±31,89^{c}	274,56±25,44^{d}	521,58±7,36^{ba}
FCR	1,45±0,05^{b}	2,41±0,20^{a}	1,66±0,18^{b}	2,28±0,36^{a}	1,44±0,06^{b}
SCR%/J	4,27±0,06^{a}	2,86±0,05^{c}	3,53±0,14^{b}	2,93 ±0,15^{c}	4,06±0,03^{a}
SR%	93,33±5,77^{a}	93,33±2,88^{a}	90±0,10^{a}	85±13,23^{a}	95±5^{a}

9 **NB**: The letters a, b, c and d show the differences between the results of the statistical analyses.

The IMW value was constant at 1.56 g for all diets (R0 to R4). This indicates that all fish groups had a homogeneous initial weight, which is essential to ensure that the differences observed in the other parameters are not due to variations in initial weight.

42 With regard to growth parameters (FMW, AMWG, RMWG, SGR), no statistically significant differences were observed between fish fed diets R0 and R4. Diets R1 and R3 showed no significant differences between them, but were both significantly lower than R0 and R4. For SGR, no significant differences were observed between fish fed diets R0 and R4, nor between R1 and R3. On the other hand, fish fed the R2 diet showed statistical differences from R0, R4, as well as R1 and R3.

In terms of feed efficiency (FCR), fish fed with R4 and R0 diets recorded the best FCR with values of 1.44 and 1.45 respectively, followed by those fed with R2 diet (1.66). However, these three diets had no significant statistical difference and were better than R1 (2.41) and R3 (2.28), which had no significant difference. On the other hand, fish fed diets R0, R2, R4 and those fed diets R1(2.41), R3(2.28) had FCR that were statistically different.

8 With regard to survival rates, the results obtained in this study do not show a significant difference for fish fed the R0, R1, R2, R3 and R4 diets. Fish fed the R3 diet had the lowest survival rate, with a value of 85%.

Economic analysis

TableVI : Economic analysis of tested diets

Parameters	R0	R1	R2	R3	R4
Cost of Kg of feed (FCFA)	1050	657,22	654,72	691,43	666,41
Cost of Kg of fish (FCFA)	1523,91±50,26[a]	1585,86±137,72[a]	1087,76±119,76[b]	1579,70±250,33[a]	958,94±39,58[b]

NB: The letters a and b show the differences between the results of the statistical analyses.

Table VI shows the economic analysis of the diets tested. For feed costs, diet R0 (industrial feed) has the highest cost (1050 FCFA), while diets R1, R2, R3 and R4 have significantly lower costs (around 657 to 691 FCFA).

Production costs per Kg of fish vary between groups, with R4 (958.94 FCFA) and R2 (1087.76 FCFA) showing the lowest costs and no significant statistical difference**.** Diets R0, R1, and R3 have the highest costs, exceeding 1500 FCFA and showing no significant statistical difference.
However, R4, R2 and R0, R1, R3 are statistically different.

III.1.6. Fish flesh analysis

TableVII : Results of bromatological analysis of fish flesh

Parameters	Initial fish	R0	R1		R3	R4
Crude Protein (%)	16,81±0,10[a]	17,09±0,46[a]	16,82±0,10[a]	16,58±0,20[a]	16,74±0,84[a]	17,05±0,76[a]
Crude Fat (%)	3,94±0,19[b]	6,40±0,43[a]	4,31±0,54[b]	4,22±0,19[b]	4,57±0,15[b]	5,70±0,24[a]

Ash (%)	3.94±0.14[bac]	3.49±0.26[dc]	4.25±0.19[ab]	4,53±0,13[a]	3.76±0.22[dc]	3,28±0,31[d]

NB: The letters a, b, c and d show the differences between the results of the statistical analyses and

Bromatological results show that crude protein remains constant between groups, with values ranging from 16.58% to 17.09%, with no significant difference. On the other hand, Crude fat content varies significantly: group R1 has the highest content (6.40%), while the other groups (R0, R2, R3 and R4) have lower values, ranging from 3.94% to 4.57%, with no significant statistical difference. In terms of ash, groups R2 and R3 stand out with higher contents (4.25% and 4.53%, respectively), indicating better mineralization. Conversely, groups R4 (3.28%) and R1 (3.49%) show the lowest values, statistically different.

III.2. Discussion

Temperatures recorded during the experiment ranged from 26.6°C to 30.1°C. These results are in line with those of Alofa *et al.* (2016), who argued that *Clarias* can withstand temperatures as low as 6°C and as high as 50°C. However, a temperature range of 28 to 30° C is considered optimal for the growth of *Clarias gariepinus* and *Clarias anguillaris* (Adebayo *et al.*, 2008).

The pH ranged from 7.11 to 8.94. These results fall within the range reported by Geoffroy et al. (2019), who summarized the literature on biological and zootechnical parameters of the African catfish *Clarias gariepinus* and argue that *Clarias gariepinus* survives and thrives best in water with a pH between 6 and 9.

In the present study, the highest growth was observed in diet R4 (a mixture of blood meal, chicken viscera meal and black soldier fly larvae meal).

Mbaye (2023) has shown that blood meal and black soldier fly larvae meal represent promising alternative protein sources in animal feed. Protein-rich blood meal and black soldier fly larvae meal were evaluated for their nutritional benefits, but it was observed that the higher the fishmeal substitution, the higher the FCR. In terms of feed efficiency

(FCR), fish fed the diet containing 5% bloodmeal recorded the best FCR. The best growth performance was also noted in fish fed the diet (5% blood meal). These results are similar to those of Olukunle *et al.* (2002), who reported that optimal growth and nutrient utilization in hybrid Clarias (male *Clarias gariepinus* x female *Heterobranchus longifilis*) were achieved with 5% sun-dried bloodmeal.

Similarly, fly larvae meal, recognized for its durability and nutritional value, has shown significant potential as an additive. Study have suggested that the optimal incorporation rate for this insect meal should not exceed 30%, due to its chitin content (Gougbedji, 2022).

In this study, the R1 diet containing 5% chicken viscera meal had the highest FCR, followed by the R3 diet containing 2.5% chicken viscera meal and 2.5% black soldier fly meal. These results concur with those of Amtul *et al*. (2012) who worked on replacing fish meal with poultry by-product meal (chicken intestine) as a protein source in carp fry diets. Diets containing chicken intestine meal, even at the lowest level, significantly limited growth performance and feed efficiency. Values deteriorated as the incorporation rate of chicken intestine meal increased. This was due to the ash and carbohydrate content of the gut meal, producing a faster intestinal transit rate, leading to increased feed intake, associated with poor growth performance and feed efficiency. Similar results were reported by Ronyai *et al*. (2002) and Fagbenro *et al.* (2004), who revealed that deficiency in one or more amino acids limits protein synthesis and affects fish growth

The R2 diet containing 2.5% blood meal and 2.5% chicken viscera meal has a FCR of 1.66, and these results are in line with those of Milliamena (2002) and Guo *et al*. (2007) who obtained better results when using blood meal to balance lysine content by replacing fishmeal with poultry byproducts (including broiler viscera) used alone or in combination with other ingredients of animal origin. According to Hu *et al*. (2008a), methionine and lysine are present in small quantities in chicken viscera (1.43 and 3.51% respectively). This could account for the low growth achieved in fish diets fed chicken viscera. The use of cow blood meal can correct lysine deficiency due to its richness in this amino acid (Hertrampf and Piedad-Pascual, 2000 and Alofa et *al.*, 2016). However, methionine deficiency remains. Although poultry by-products such as chicken viscera have proven to be nutritionally adequate protein sources for many fish species (Fowler, 1991; Robaina *et al.*,1997; Abdel-Warith *et al.*, 2001; Wang *et al.*, 2006), they were deficient in essential amino acids such as lysine, methionine that limited their utilization (Glencross et *al*., 2007). Therefore, it will be beneficial to adjust the level of essential amino acids in chicken viscera through supplementation. Hu et *al.* (2008b) provided 0.49% lysine and 0.14% methionine as supplementation in diets containing chicken viscera with improved growth performance in *Carassius auratus gibelio*. Similar trends in results were obtained by Rawles et *al*. (2006) in the *Morone chrysops* x *Morone saxatilis* hybrid fed a broiler viscera diet supplemented with methionine (0.63%) and lysine (1.55%)

The results of this study show that the diet containing a mixture of chicken viscera meal, cow blood meal and black soldier fly meal, with a total incorporation rate of 5%, offers the best growth performance for catfish hybrid fry *of Clarias gariepinus* and *Clarias anguillaris*. This success can be attributed to the nutritional complementarity of the three ingredients: chicken viscera meal, rich in digestible proteins and deficient in amino acids including lysine and methionine according to Wang et *al.* (2006), cow blood meal which provides iron-rich proteins beneficial to hematopoiesis (Olukunle *et al.*, 2002) and black soldier fly meal, a sustainable source of essential fatty acids and balanced proteins (Gougbedji, 2022; Henry et *al.*, 2015). The synergistic effect observed with this blend, highlighted by Santiago and Lovell (1988) and Youssouf and Sedro (2020), probably results from the combination of diversified nutritional inputs that optimize nutrient digestibility and assimilation. By keeping the incorporation rate low (≤ 5%), negative effects such as reduced palatability or digestibility were avoided, as noted by El Sayed et *al.* (1998). These results also confirm the findings of similar study done by Youssouf and Sédro (2020) who reported that diets combining different animal and insect protein sources improve growth performance and feed conversion in fish.

The results in Table VIII highlight the economic efficiency of treatments R4 and R2, characterized by significantly lower production costs per Kg of fish, respectively 958.94 and 1087.76 F CFA compared to R0, R1 and R3, where these costs exceed 1500 FCFA. These data corroborate the studies by Tacon and Metian (2015), who emphasized the importance of optimized feed formulation to reduce production costs in aquaculture, by favoring accessible and digestible ingredients. Furthermore, the discrepancy observed between treatments may be explained by variations in feed conversion rate (FCR), a key parameter influenced by feed composition and rearing conditions, as suggested by Boyd and Tucker (2012). Thus, the results reinforce the idea that the use of adapted formulations (R4 and R2) can improve profitability while reducing the economic and environmental impact of fish production.

Results for overall fish body composition showed similar protein content in all treatments.

However, lipid content increased, probably due to the level of chicken viscera meal in the diets. These results are in agreement with Goda et *al.* (2007), Giri et *al.* (2010) and Sugumaran et *al.* (2015), who reported that replacing fish meal with poultry viscera meal in the diets did not affect the body protein content, but increased the whole-body lipid content of the fish.

Conclusion

This study is part of an innovative approach to evaluate the effects of cow blood meal, chicken intestine meal and black soldier fly larvae meal as additives in the feed of hybrid fry *of Clarias gariepinus* and *Clarias anguillaris*. The results obtained show that these ingredients, used together at a total incorporation rate of 5%, offer significantly better growth performance and feed efficiency than the other diets tested.

Diets containing the mixture of these three ingredients were well accepted by the fish, with no pathological symptoms or negative impacts on their health. Environmental conditions, notably temperature and pH, were favorable throughout the experiment. These observations underline the potential of these raw materials as viable and sustainable alternatives to conventional fishmeal.

In order to consolidate the results obtained in the present study, it would be relevant to analyze the impact of the mixture on the fish immune system and disease resistance, and

26 to study the long-term effects of these additives on the health and overall performance of fish in different production systems.

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