

Effects of the substitution level of soybean meal by *Chromolaena odorata* leaves powder on the survival rate and growth characteristics of *Clarias jaensis* (Boulenger, 1909) juveniles in

ponds

Comment [MOU1]: The feeding trial in pond or tank?

ABSTRACT

Aims: The aim was to contribute to the improvement of *Clarias jaensis* productivity through the evaluation of the effect of the substitution level of soybean meal by *Chromolaena odorata* leaves powder on the survival rate and growth characteristics of *Clarias jaensis* juveniles.

Place and duration of the study: From March to June 2019 at TAWON (LN: 5°10' - 5°20' and LE: 10°20' - 10°21'). Sudano-Guinean altitude zone of West Cameroon.

Study Design: Two hundred and twenty-five *Clarias jaensis* juveniles (12.44 ± 0.15 g) were divided into five treatments corresponding to 0; 10; 15; 20 and 25% of substitution level of soybean meal by *Chromolaena odorata* leaves powder.

Methodology: They were fed twice a day (8:00 A.M. and 6:00 P.M.) at 5% of ichthyobiomass, total length, live body weight and physicochemical parameters were measured every three week for 84 days.

Results: At the 15% substitution level of soybean meal, the survival rate was the highest in 15% (100%). Mean Final Weight and Mean Daily Weight Gain were significantly ($P \leq 0.05$) higher at 15% (45.73 ± 7.45 g and 0.56 ± 0.31 g/d). Specific Growth Rate and Condition factor K were higher at 25% ($1.41 \pm 0.68\%$ /d and $1.08 \pm 0.22\%$ /cm³), however, no significant differences were recorded. The cost of feed to produce one kg of *Clarias jaensis* was lower at 15% (609.61 Fcfa).

Conclusion: Soybean meal can be substituted by *Chromolaena odorata* leaves powder at 15% in feed for *Clarias jaensis* juveniles.

Keys words: *Chromolaena odorata*, *Clarias jaensis*, Feeding, growth, leaves powder.

1. INTRODUCTION

Fish is one of the most widely consumed foods in Africa [1]. This is due to the fact that it is found in everyday meals, as it is the main source of animal protein for poor populations [2]. It thus contributes around 50% of GDP in animal protein intake in Sub-Saharan Africa [3] and covers almost 50% of people's demand for animal protein in Cameroon. With the national population growing exponentially and urbanization on the rise, national demand for fish products has increased considerably in recent years. It rose from 409519 tons in 2013 to an average of 433616 ~~tonstons~~ in 2015 [4]. Currently, forecasts for 2020, for the increase in demand are estimated at 496891 ~~tonestons~~. However, fishing alone is no longer sufficient to sustainably satisfy the ever-increasing demand, due to overfishing, climate change, disruption of the community balance and environmental degradation [3]. Faced with this drastic situation, aquaculture is becoming an indispensable alternative for fish production, but the implementation of this activity faces several constraints, including the lack of high-performance feed on the market at prices affordable to fish farmers, and the unavailability of quality fry.

In aquaculture, feed represents around 50% of the cost of producing a kilogram of fish. Soybean meal is one of the most widely used ingredients in fish feed formulation [3]. However, it represents 49.0% of total protein in standard feeds for fish farming [5] -and is the most widely used element in animal feed after cereals. Its scarcity, high market price, and competition have led researchers to look for new sources of protein of plant origin, particularly those that are not consumable by humans [6, 7].

As soybean meal sold in Cameroon only comes from imports, it is very difficult for fish farmers with modest incomes to access, so it will be necessary to resort to alternative sources of protein, particularly plant-based [8,9,10]. These plant-derived raw materials are interesting sources of protein, and are known to successfully substitute proteins derived from animal meal and soybean meal in several fish species [11, 12, 13, 14 and 15]. *C. fromolaena odorata* meal used in animal production [16] could be a source of protein in fish feed in general, and *C. larias jaensis* in particular. A study was carried out on the effect of substituting soybean meal with *C. fromolaena odorata* leaf powder on the survival and growth characteristics of *C. larias jaensis* juveniles. In intensive aquaculture, feed represents a significant proportion of fish production costs [17]. The economic interest of this type of farming is therefore highly dependent on the availability and cost of feed [6, 18]. For this reason, reducing feed-related costs, and consequently lowering the total cost of fish production, is one of the priorities in aquaculture [19]. Moreover, soybean meal

is the most important ingredient in terms of digestible protein content when formulating balanced diets for aquaculture. Indeed, it is rich in Essential Amino Acids (EAA), whose profile corresponds remarkably well to the needs of farmed fish [17]. However, its high purchase price, competition with other crops, and human consumption of soya mean that access to it is very limited for small-scale producers. As a result, aquaculture research needs to find alternative sources of protein, particularly of plant origin, which are not directly usable for human consumption [8, 9] and which can provide fish with the elements they need for growth. The aim of this work is This work aims to contribute to the improvement of *C. larius jaensis* fish farming productivity through the use of *C. bromolaena odorata* leaves in fish feed.

2. MATERIALS AND METHODS

2.1 Study area

The study took place from February to June 2020, on the farm of the Common Initiatives group at Tawoum (GIC PIAT) located in the subdivision of "Santchou", Menoua Division, West Cameroon Region (LN: 5°10' - 5°20' and LE: 10°20' - 10°21') in the highland Sudano-Guinean zone of West Cameroon [20].

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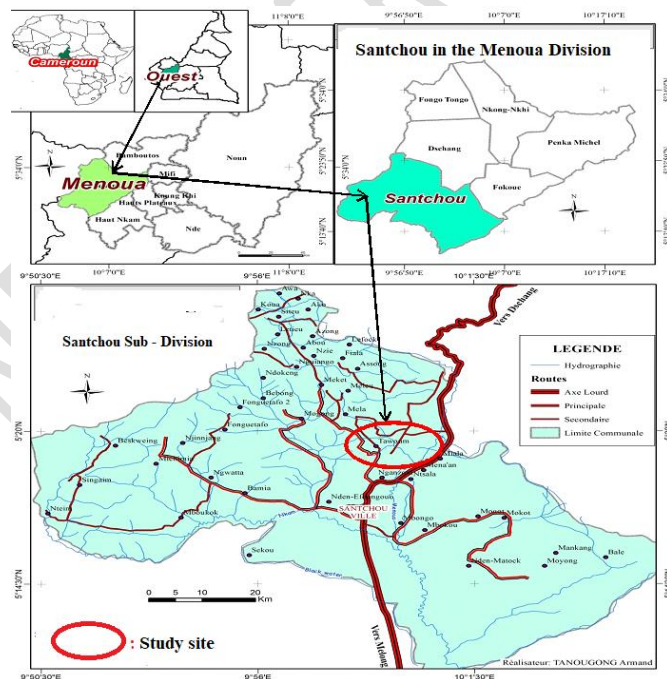


Figure 1: Location of the study site.

2.2 *Chromolaena odorata* leaves powder preparation

The *Chromolaena odorata* leaves were harvested from abandoned plantations and roadside verges. These leaves were dried in the sun on corrugated iron sheets for three days and then ground using a hammer mill. The dried leaves were finely ground and sieved through 400 micrometer mesh (Figure 2).



Figure 2: *Chromolaena odorata* leaves and powder leaves

2.3 Experimental Diets Formulation

The conventional ingredients used in this process were fish meal, soybean meal, cottonseed cake, maize, palm kernel meal and groundnut cake, which were obtained sourced from the local market. The ingredients were weighed according to ~~table~~Table 1. ~~The ingredients were and~~ blended until a homogeneous powder was obtained, to which vegetable oil was added. Water was then added at a rate of 60% dry matter, to form a malleable paste which, passed through the die of a meat mincer (TC 22SL), yields 3 mm diameter filaments (spaghetti). These filaments were then sun-dried, broken down to the desired size, bagged and stored until distribution to fish juveniles.

Table 1: Composition of the different formulated feeds treatments

Ingrédients	Feed treatments (% of substitution of soybean meal by <i>Chromolaena odorata</i> leaves powder-)				
	0	10	15	20	25
Corn	11	11	11	11	11
Wheat bran	10	10	10	10	10
Cottonseed cake	10	10	10	10	10

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Soybean meal	30	27	25.5	24	22.5
Peanut meal	5	5	5	5	5
Palm kernel cake	5	5	5	5	5
Fish meal	26	27	27	27	27
<i>C. chromolaena</i>	0	3	4.5	6	7.5
<i>odorata</i>					
Palm oil	2	2	2	2	2
Premix	4	4	4	4	4
Total	100	100	100	100	100

2.4 Experimental fishes

A total of 225 *Clarias jaensis* fry of average weight 12.44 ± 0.15 g were collected from local fishermen, and fed from April to June 2020 with five iso-protein feeds formulated with *C. chromolaena odorata* leaves powder.

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2.5 Experimental Design

Five experimental feeds were manufactured at 40% crude protein, substituting soybean meal with *Chromolaena odorata* leaves powder, at the rate of 0; 10; 15; 20 and 25%.

2.6 Feeding trials

These feeds were tested on *Clarias jaensis* juveniles, with a mean initial weight of 12.44 ± 0.15 g. A batch of 225 juveniles was individually weighed and randomly distributed in 15 tanks of 0.04m^3 usable volumes, i.e. 15 juveniles per tank, thus forming five (5) triplicate treatments, each corresponding to the different diets.

The fishes were not fed until the day after distribution, to reduce stress due to handling during loading. Each ration was distributed half and half twice a day (8 and 18 hours). The quantity distributed per day represented 5% of the ichthyobiomass [21, 22], readjusted every 21 days after the control fishing. The trial lasted 84 days.

Every 21 days, fishing was carried out to monitor the various zootechnical parameters, and 33.33% of the population in each batch was weighed using a 1.01 g precision balance and measured using a 30 cm graduated ruler. The entire population was counted to determine survival rate.

Water temperature was recorded twice daily (6.30 a.m. and 5.30 p.m.) using a thermometer. Water pH and transparency were measured once a week on the same day, using pH strips and a specially designed Secchi disc respectively.

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2.7 Characteristics studied

- Survival rate (SR)

$$SR = \frac{\text{Final Number of fishes}}{\text{Initial Number of Fishes}} \times 100$$

- Mean Weight Gain (MWG)

$MWG (g) = FMW - IMW$ Where IMW = Initial Mean weight (g) and FMW = Final Mean weight (g)

- Specific Growth Rate (SGR)

$$SGR = \frac{\ln(FMW) - \ln(IMW)}{T_2 - T_1} \times 100$$

Where ln = Neperian logarithm and $T_2 - T_1$ = Experimental period (days)

- Average Daily Mean Weight Gain (ADMWG)

$$ADMWG (g) = \frac{FMW - IMW}{T_2 - T_1}$$

- Feed Conversion Ratio (FCR)

$$FCR = \frac{\text{Dried Feed Intake}}{MWG}$$

- Condition factor K

$$K = \frac{W (g)}{LT (cm)^3} \times 100$$

Where W = weight (g), LT = Total Length (cm)

2.8 Statistical analysis

One-way analysis of variance (ANOVA) was used to test the effect of the level of substitution of soybean meal by *Chromolaena odorata* leaf powder on the survival rate and growth characteristics of *Clarias jaensis* juveniles. Where differences existed, Duncan's test was used to separate means at the 5% threshold. SPSS 20.0 (Statistical Package for Social Sciences) was used for the various analyses.

3. RESULTS

3.1 Survival rate and growth characteristics of juveniles of *Clarias jaensis* as a function of the level of substitution of soybean meal by *Chromolaena odorata* leaf powder

The Table 2 shows the overall results of survival rate and growth characteristics of *Clarias jaensis* juveniles.

Table 2: Variation in survival rate (%) and growth characteristics as a function of soybean meal substitution level by *Chromolaena odorata* leaf powder

Survival rate and growth characteristics	Substitution level's (%)					P
	0 (n=10)	10 (n=10)	15 (n=10)	20 (n=10)	25 (n=10)	
SR (%)	98,3±3,3 ^a	100 ^a	100 ^a	98,3±3,3 ^a	96,7±6,7 ^a	0,413
IMW(g)	12,27±1,98 ^a	12,4±1,54 ^a	12,6±2,09 ^a	12,33±1,75 ^a	12,60±1,99 ^a	0,982
FMW(g)	45,53±5,65 ^a	44,6±6,04 ^a	45,73±7,45 ^a	33,33±2,94 ^b	32,47±3,13 ^b	0,000
MWG(g)	11,87±5,54 ^a	11,47±5,21 ^a	11,93 ±6,45 ^a	6,87±3,44 ^b	7,27±4,80 ^b	0,009
DMWG (g/j)	0,56 ± 0,26 ^a	0,54 ± 0,24 ^a	0,56 ± 0,31 ^a	0,32 ±0,16 ^b	0,34 ±0,22 ^b	0,009
SGR (%/d)	1,41 ± 0,59 ^a	1,38 ± 0,56 ^a	1,41 ± 0,68 ^a	1,11 ±0,57 ^a	1,21 ±0,80 ^a	0,612
K	1,07 ± 4,12 ^a	1,04 ± 4,06 ^a	1,08 ±0,22 ^a	0,86 ±0,18 ^a	0,83 ±0,12 ^a	0,250
FCR	1,84±0,43	2,05 ±0,95	1,86±0,46	2,46 ± 0,70	2,89 ± 1,65	

SR: Survival rate, IMW: Initial Mean Weight, FMW: Final Mean Weight, MWG: Mean Weight Gain, DMWG: Daily mean Weight Gain, SGR: Specific Growth Rate, K: Condition factor, FCR: Food Conversion Ratio.

(a, b): on the same line, values affected by the same letter do not differ significantly ($P>0.05$). (n): number of observations.

The highest survival rates were recorded in batches fed with 10% and 15% of substitution level of soybean meal by *Chromolaena odorata* leaf powder, and the lowest in 25%. However, there were no significant differences ($P>0.05$).

The Final Mean Weight, Mean Weight Gain and the Daily Mean Gain were significantly higher at 15% of substitution level of soybean meal by *Chromolaena odorata* leaf powder. However, they were significantly different from those obtained with 20% and 25%. On the other hand, the specific growth rate and condition factor K recorded the highest values at 15% and the lowest value was at 25% of substitution level of soybean meal by *Chromolaena odorata* leaf powder, but the statistical analysis shows no significant difference ($P>0.05$).

3.2 Daily evolution of growth characteristics of juveniles of *Clarias jaensis* with the soybean meal substitution level by *Chromolaena odorata* leaf powder

3.2.1 Mean Weight

The Figure 3 shows the evolution of the Mean Weight of *Clarias jaensis* juveniles with the

soybean meal substitution level by *Chromolaena odorata* leaf powder.

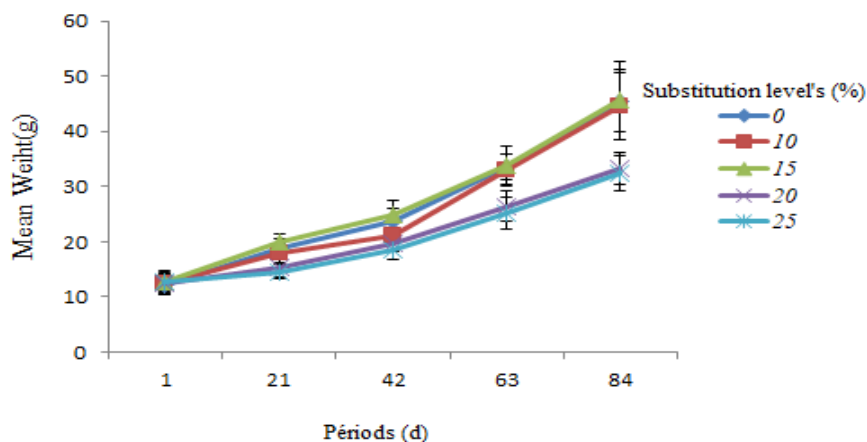


Figure 3: Daily evolution of the Mean Weight of juveniles of *Clarias jaensis* as a function of the of substitution level of soybean meal by *Chromolaena odorata* leaf powder

The weight increased steadily throughout the period of study, at the end of the study, the highest values were recorded in the treatments receiving 20% and 25% of the substitution of soybean meal by *Chromolaena odorata* leaf powder. These results show that growth remained lower in the treatment receiving 25% *Chromolaena odorata* substitution.

3.2.2 Mean Daily Weight Gain

The Figure 4 illustrates the evolution of mean Daily Weight Gain as a function of the level of substitution of soybean meal by *Chromolaena odorata* leaf powder.

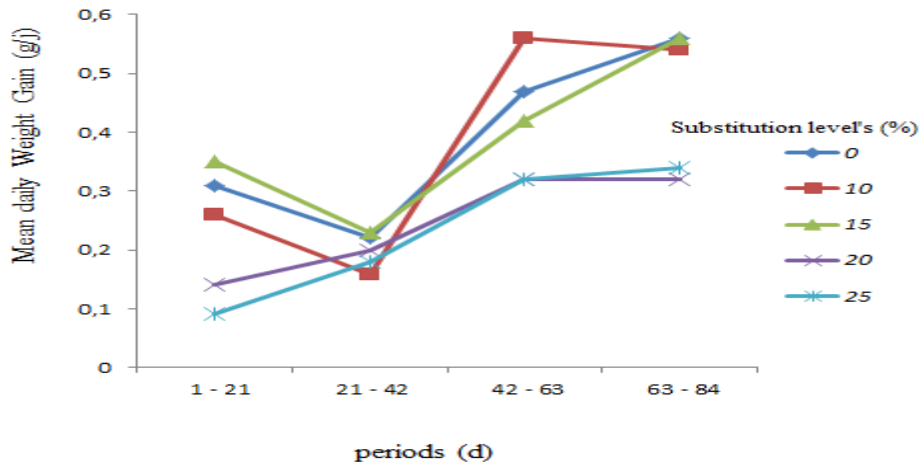


Figure 4: Evolution of Mean Daily Weight Gain as a function of the level of substitution of soybean meal by *Chromolaena odorata* leaf powder.

Overall, the figure shows that the mean Daily Weight gain decreased throughout the first three weeks of the trial, with the exception of treatments 20% and 25%, which showed an increase. In the sixth week, however, it increased in all treatments. At the end of the trial, the highest values were recorded at 10% and 15% of substitution of soybean meal by *Chromolaena odorata* leaf powder.

3.2.3 Condition factor K.

The daily evolution of condition factor K of juveniles of *Clarias jaensis* as a function of the substitution level of soybean meal by *Chromolaena odorata* leaf powder is shown in figure 5.

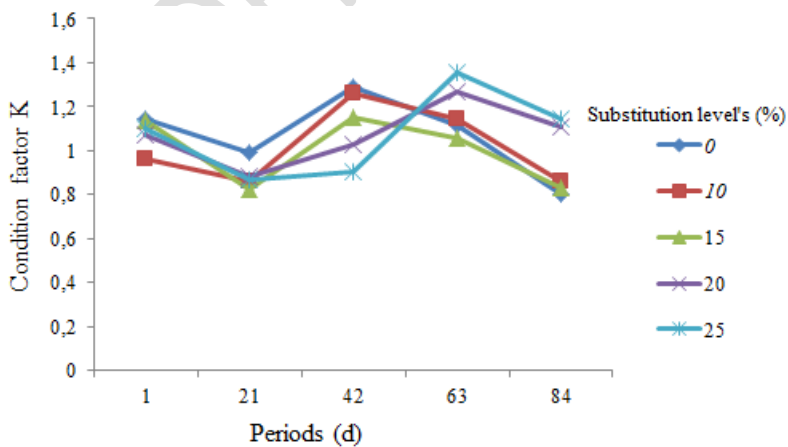


Figure 5: Daily evolution of Condition factor K as a function of the level of substitution of soybean meal by *Chromolaena odorata* leaf powder.

The profile and pacing are comparable between the levels substitution of *Chromolaena odorata* on all days; at the end of the trial, no significant differences were found ($P>0.05$).

3.2.4 Feed cost of producing one kg of fish *Clarias jaensis*

The table 3 summarizes the cost of one kg of feed and the feed cost of producing one kg of fish.

Table 3: Food cost of producing one kg of *Clarias jaensis*

Substitution level's (%)	Cost of one kg of feed (Fcfa)	FCR	Feed cost of one kg of fish (Fcfa)
0	346,25	1,84	637,10
10	335,25	2,05	687,26
15	327,75	1,86	609,61
20	320,25	2,46	787,81
25	302,75	2,89	874,94

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The table shows that feed costs decreased with the substitution level of *Chromolaena odorata*. The lowest value was obtained with 15% and the highest was at 25% of substitution level of *Chromolaena odorata*.

3.3 Discussion

The survival rates were high in all treatments. The lowest survival rate (96.7%) was recorded in batches receiving treatment 25% of substitution of Soybeansoybean meal with the leaves powder of *Chromolaena odorata* and the highest values (100%) were recorded in batches receiving treatments 10% and 15%. These results are higher than those of *Clarias gariepinus* fingerlings [23] at the end of eight weeks of rearing, which was 75% with a feed containing 20% protein at a temperature varying between 27.5 and 28°C, with a pH between 7.5 and 7.8. This difference was due to the physico-chemical conditions of the water obtained throughout the trial period. On the other hand, these values are comparable with 100% in *Clarias gariepinus* juveniles fed with Néré almond meal, and 94.87 to 100% in those fed with soybean [24].The highest Final Mean

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Weight value (49.47g) recorded in the present study for animals fed for 84 days was lower than that obtained with *Clarias gariepinus* juveniles with an initial weight of 13.1 g and fed with isoprotein experimental feed (40% protein), was 62 g [24]. ~~This~~These differences could be explained by the quality and content of the anti-nutritional factors contained in *Chromolaena odorata* leaves. The highest Mean Daily Gain (0.56g/d) obtained with 0% and 15% was significantly ($P < 0.05$) lower than 3 g/d with *Clarias gariepinus* [25]. However, it remains higher than the result with *Moringa oleifera* leaves (0.19 g/d) in *Clarias gariepinus* [26]. This suggests that *Chromolaena odorata* may be a good alternative protein source for feeding *Clarias jaensis*. The lowest specific growth rate (1.21%g/d) obtained in this study remains higher than that obtained at Fouban fish farm, which ranged from 0.04 to 0.18%g/d [27]. This result is comparable to 1.9%g/d in *Clarias gariepinus* [6]. However, it is still lower than 4.78 to 5.8%/d [28] and 3.85 - 4.26 %/d respectively obtained for fish fed with feed containing 0, 30, and 50% Azolla [28] and 3.60%/d in juvenile *Clarias gariepinus* [24]. This could be justified by the density and size of the fish used by the latter in their trials. The lower SGR values in this study could be explained by the tannins contained in *Chromolaena odorata* leaves. Indeed, tannin is more concentrated in leaves than in stems [30]. This high tannin content can reduce the palatability and nutritional value of the feed. As aquaculture feeds are richer in protein than those for other farmed animals, the reduction or elimination of these factors is important if plant raw materials are to be effective as ingredients in fish feeds [31]. It will therefore be essential to check the tannin content of *Chromolaena odorata*, as anti-nutritional factors in general, and tannin in particular, present in varying quantities depending on the plant product, can intervene at different levels such as apprehension, digestion of proteins and other nutrients or metabolism, as enzyme inhibitors or as anti-vitamins [32, 33, 34]. The mechanisms of action of most of them are not known in fish. Condition factor K varied with treatment. Values obtained for *Clarias jaensis* ranged from 0.83 to 1.08. These values were comparable to 0.62 - 1.86 reported in *Protopterus aethiopicus* [35]. Nevertheless, it is higher than 0.79 - 0.83% in *Clarias gariepinus* [36] fed with complete feed and 0.06 - 0.74 [37]. The differences between these values would be linked to the optimal use of plant resources in breeding and species. With regard to the feed conversion ratio (FCR), the values found in this study (1.84 - 2.89) are very high compared with 1.95 - 2.08 [38] and 1.51 - 2.11 [17]. This could be explained by the strong odor emitted by *Chromolaena*

odorata. The food containing 15% of the *Chromolaena odorata* substitution level had the lowest feed cost, indicating that it was the most economically efficient.

4. Conclusion

At the end of this study, which focused on the effect of the level of substitution of soybean meal by *Chromolaena odorata* leaves powder on the survival and growth of *Clarias jaensis* juveniles, and whose main objective was to contribute to the improvement of *Clarias jaensis* fish farming productivity through the use of *Chromolaena odorata* leaves as food ingredient, the following main conclusions were obtained: The survival rate of *Clarias jaensis* juveniles was not significantly affected ($p > 0.05$) by the substitution level of *Chromolaena odorata*. However, the highest survival rates were recorded in batches fed with treatments 10% and 15%. Growth characteristics of *Clarias jaensis* juveniles such as Mean Weight Gain and Mean Daily Weight Gain were significantly affected ($p < 0.05$) by the substitution level of *Chromolaena odorata*, while Specific Growth Rate and Condition factor K were not affected. However, it should be noted that the best growth characteristics were obtained with treatments 15% substitution level of the *Chromolaena odorata*. In terms of feed cost, 15% substitution level of the *Chromolaena odorata* showed the best production cost.

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