Evaluation of the digestibility of diets containing soy okara in *Clarias gariepinus* (Burchell, 1822) fries raised in a controlled environment in Benin

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ABSTRACT

Aims: To evaluate the digestibility of diets containing soy okara in *Clarias gariepinus* fries
Study design: Random complete block design.
Place and Duration of Study: Laboratory of Hydrology and Wetland Research, Faculty of Sciences and technics, University of Abomey-calavi, between June 2019 and July 2019.
Methodology: Four diets containing different levels of soy okara (0%, 10%, 20% and 30%) respectively noted T_0, T_1, T_2 and T_3 were tested. Twelve 40L tanks were randomly distributed into 04 batches with 03 repetitions for each of the 04 diets. The frieswith an initial average weight of 10.47+/-2g were fed *ad libitum* three times a day (8 A.M., 12 P.M., 4 P.M.) for 30 days. Survival rate, cannibalism rate, average daily gain, specific growth rate, weight-length relationship, condition factor, consumption index, ingested protein, protein efficiency coefficient and digestive utilization coefficients were measured.
Results: The specific growth rates (SGR) ranged between 2.25+/-0.165 and 2.58+/-0.115 with the best rate for the treatment containing 0% soy okara. The consumption index varied from 1.18+/-0.009 to 1.41+/-0.132 with the best index for the T_3 treatment. Furthermore, the mortality rates were 0% for treatments containing 20% and 30% soy okara. The results indicate that it is possible to incorporate up to 30% soybean okara into the diet of *Clarias gariepinus* fry without hindering their zootechnical performance. The digestive protein utilization coefficients of the diets were calculated and are 95.12; 88.47; 92.21 and 90.37 for diets containing 0%, 10%, 20% and 30% soy okara respectively.
Conclusion: The results demonstrate that among the different diets tested, treatment T_2, containing 20% soy okara, proved to be the most favorable in terms of zootechnical performance. These results suggest that T_2 treatment can be considered as a promising option for feeding *Clarias gariepinus* fries.

Keywords: Clarias gariepinus, soy okara, feeding, growth, digestibility, food waste, water pollution.

1. INTRODUCTION

Recent trends around the world point to a decline in capture fisheries landings, indicating that fish stocks have reached or even exceeded the point of maximum yield (FAO, 2017). Faced with this situation, aquaculture becomes the only viable alternative for increasing fish production with the aim of satisfying the protein needs of the population. The promotion of the aquaculture sector requires, among other things, the reduction of production costs which have until now remained exorbitant and mainly influenced by the cost of feed. To achieve

this, several attempts to reduce food costs have been noted through several research and development projects. Thus, several by-products or agricultural products with low commercial values but rich in nutrients have been incorporated into fish feed with the intention of improving their zootechnical performance. This is the case, for example, for seeds of *Leucaena leucocephala*[1], leaves of *Moringa oleifera*[2], cotton seeds [3], azolla [4,5] etc. which, when incorporated into foods, showed appreciable results on the growth of *Oreochromis niloticus* and *Clarias gariepinus*. However, some limitations can be noted regarding these practices. Indeed, the use of these ingredients poses problems of availability and cost. To overcome these obstacles, there is an urgent need to find sources of protein and nutrients that are available on the local market and that are of low commercial value. This is the case, for example, of soy okara obtained after the manufacture of soy cheese which is increasingly available across the country and whose cost is sufficiently low.
Furthermore, controlling the incorporation rates of agricultural by-products constitutes another problem facing fish farmers and researchers. Indeed, in Benin, several studies have been carried out in several research centers to determine the optimal incorporation rates of certain by-products used in fish farming. However, none of them focused on the actual digestibility of these products in these aquaculture species. Digestibility, which is the quantification of digestive processes, provides relative information on the extent to which ingested foods and their nutrient components are digested and absorbed by the animal [6]. Apparent digestibility coefficients vary depending on fish species and raw materials. It is therefore important to determine the digestibility of feed materials, not only to enable the formulation of feeds that improve the growth of farmed fish, but also to limit waste produced by fish. Going further, it is important to determine the digestibility of individual ingredients in compound diets, which is considered one of the important factors that can improve fish growth [6]. It is in this context that we focused our attention on soy okara which is a rich source of protein [7]. The digestibility of this by-product was evaluated by incorporating it alone and by combining it with chicken viscera in the diet of *C. gariepinus* fries at the Laboratory of Hydrology and Wetland Research (LHyReZ). This study entitled “Evaluation of the digestibility of diets containing soy okara in *Clarias gariepinus* fries raised in a controlled environment” aimed to better understand the potential use of this ingredient. At the same time, it made it possible to propose a solution approach for improving the quality of food for *C. gariepinus* fries and reducing food waste. It thus made it possible to preserve the quality of the environment in general and of the breeding water in particular and therefore to reduce tasks and production costs for fish farmers.

2. METHODOLOGY

2.1 Formulation of experimental foods

The main raw materials used in feed formulation are fish meal, soy okara, chicken viscera, soy meal, cassava starch, corn flour, cottonseed meal, methionine, peanut oil, mineral and vitamin premixes. To study digestibility, chromium oxide (Cr_2O_3) was added to each diet at a rate of 0.5% as an inert marker. The floury ingredients were then mixed until a homogeneous paste was obtained by adding 53.33% boiling water to make the different diets. The homogeneous paste was finally formed into 2mm diameter granules using a mixer granulator. Four experimental diets T_0, T_1, T_2 and T_3 containing 0%, 10%, 20% and 30% soy okara, respectively, were formulated (Table 1). Pellets made from these different food formulas were dried under the sun for 8 hours then stored in plastic containers until use.

Table 1. Centesimal compositions of diets

INGREDIENTS (kg)	T_0	T_1	T_2	T_3	Methods
Fishmeal	45	30	30	30	Weighing
Soy flour	10	6	3	0	

Soy Okara	0	10	20	30	
Corn flour	15	15	12	9	
Cottonseed cake	20	15	12	9	
Chicken viscera	3	16	15	14	
Peanut oil	3	3	3	3	
Cassava starch	2	2	2	2	
Methionine	0	1	1	1	
Premix min and live	2	2	2	2	
TOTALS	**100**	**100**	**100**	**100**	
Protein(%)	67.73	46.27	52.22	45.69	Bradford method
Lipid(%)	16.5	13.1	12.8	12.9	Soxhlet extraction

2.2 Experimental device

The test was carried out in circular plastic tanks of 40 liters volume, supplied with drilling water and half filled. In total, 240 *C. gariepinus* fries with an average weight of 10.47±2g were distributed among 12 tanks, i.e. 20 fries per tank. The tanks were randomly distributed into 04 batches with 03 repetitions each for the 04 diets. The fries were fed ad libitum three times a day (8 A.M., 12 P.M., 4 P.M.) for 30 days with the different diets. The different quantities consumed of each diet were determined after each feeding. The rearing water was 100% renewed every 48 hours after it was introduced into the tanks in order to recover the faeces. The water collected from each tank was stored in a plastic bucket then left to stand until it settled. The submergent was then discarded and the remainder was poured into a 15-micron sieve then the faecal concentrate was collected and centrifuged at a frequency of 1000 rpm for 15min. This faecal concentrate was stored in a plastic box for each bin and stored in the freezer until drying in a freeze dryer. The lipid and protein contents were then determined by Soxhlet extraction and by the Bradford method respectively. The physicochemical parameters, namely temperature, pH, dissolved oxygen and nitrogen compounds (ammonia, nitrate, nitrite) were recorded throughout the experiment with a thermometer, a pH-meter and an oximeter. HANNA brands and test kits respectively. Control fishing was done every week. During fishing, all fish were caught by dip nets, counted and weighed.

2.3 Zootechnical and digestibility parameters

The following zootechnical parameters were determined:

- **Survival rate:**

SR(%) = 100*Nf/Ni] where

Ni: Initial number of fish and Nf: Final number of fish.

- **Cannibalism Rate**

(CR = Number of dead mutilated fish + Number of missing fish/ Initial number of fish)

- **Average Daily Gain:**

[ADG = (Bf – Bi)/dt]

Bi: Initial biomass, Bf: Final biomass, dt: duration of the experiment

- **Specific growth rate:**

[SGR (%/d) = 100*(ln Pf – ln Pi)/dt]

- **Weight-length relationship**

There is a relationship between weight (P) and length (L), expressed by the following relationship: Pt = aLtb

The value of b determines the type of growth of a given population.

If: b = 3, growth is isometric

b>3, growth is positive allometric

b<3, growth is negative allometric

- **Condition factor**

[K = 100*Pt/Ltb], where Pt = total weight, Lt = total length in cm, b = allometry coefficient

- **Consumption index:**

[CI = Qi / (Bf- Bi)] where Qi: quantity of food ingested.

- **Ingested Protein (IP).**

IP (g) = Total weight of food distributed × Protein level of the food

- **Protein Efficiency Coefficient (PEC).**

It indicates the weight gain per unit of protein consumed, giving a measurement that determines whether the protein source of the food adequately meets the requirements of the species.

PEC = (Bf – Bi) / IP

- **Digestive utilization coefficients (DUC)**

The fish were sampled after feeding to measure the protein in their carcasses. They were therefore dried in a freeze dryer, ground and then analysed by spectrophotometry. After laboratory analyses, Digestive Utilization Coefficients (DUC) were calculated in order to quantify digestibility. The apparent DUC was used because the endogenous faecal fraction is very low in fish.

The digestibility of a nutrient was obtained according to the relationship (Cho & Kaushik 1990 cited by Mohamad-Zulkifli *et al*., 2019):

DUCa of nutrient = $\mathbf{100} - \left(\mathbf{100}\frac{\%\,\mathbf{marqueuraliment}}{\%\,\mathbf{marqueurf\grave{e}ces}} * \frac{\%\,\mathbf{nutrimentf\grave{e}ces}}{\%\,\mathbf{nutrimentaliments}}\right)$

2.4 Statistical analyses

Data were expressed as mean ± standard deviation. The data were analysed by one-way ANOVA at the threshold α = 0.05. Multiple comparisons of means were carried out with the Duncan test. Analyses were considered significant at *P*= .05. Statview software was used for statistical analyses.

3. RESULTS

3.1 Physico-chemical parameters of water

The physicochemical parameters of the water were measured in the tanks during the experiment (Table 2). Parameters fluctuated significantly (p <0.05) between tanks for pH and oxygen while temperature did not.

Table 2. Average values of physicochemical parameters

	T (°C)	O_2 (mg.l^{-1})	pH
T_0	30.99±0.25	1.86±0.22^{a}	5.58±0.03^{a}
T_1	31.00±0.46	1.86±0.16^{a}	5.50±0.05^{abc}
T_2	31.20±0.63	1.98±0.14^{ab}	5.50±0.06^{bc}
T_3	30.87±0.62	2.27±0.09^{b}	5.42±0.01^{d}
P	0.8876	0.0399*	0.0082*

Values represent mean ± standard deviation; Values in the same line that carry the same exponent are not significantly different ($p > 0.05$); the sign (*) indicates that the differences are significant ($p < 0.05$).

3.2 Weight-length relationship of fish

Table 3 presents the characteristics of the linear regression curves between individual fish weight and length for the different treatments. There is a correlation between total weight and total length when R is greater than 0.6.

Table 3. Variation of the coefficients of the weight-length relationship

	T_0	T_1	T_2	T_3
Average weight (g)	17.20±2.06	16.33 ±0.59	15.75 ±1.10	15.73 ±1.32
Total length (cm)	[8-18.2]	[11-18.2]	[9-18.8]	[10-17]
HAS	0.029	0.017	0.02	0.019
b	2,471*	2,655*	2,580*	2,602*
r^2	0.936	0.943	0.913	0.911

The sign (*) indicates that the value is significantly different from 3; a is the intercept of the regression line; b or regression coefficient is the slope of the regression line; r^2 is the coefficient of determination.

3.3 Fish condition factor K

The condition coefficient varied from 1.80 ± 0.10 (T_1) to 3.14 ± 0.81 (T_0). Statistical analyses show a significant difference ($p < 0.05$) between the condition coefficients obtained.

Table 4. Condition coefficients K

	K	Standard error
T_0	3.14±0.81	1.20
T_1	1.80±0.10	0.34
T_2	2.11±0.60	0.22
T_3	2.03±0.43	0.51
P	*< 0.0001**	

The sign (*) indicates that the differences are significant at the 5% level.

3.4 Zootechnical and feed utilization performance

Zootechnical and feed utilization parameters were calculated and are presented in Table 5. No significant differences were observed between treatments at the 5% threshold for ADG, SGR, cannibalism rate and consumption index. The protein efficiency coefficient PEC on the other hand varied from 1.26±0.01 (T_0) to 1.77±0.01 (T_1) and the statistics reveal a significant difference at the 5% threshold. In general, the T_2 treatment offers the best results with its null mortality rate combined with the other parameters.

Table 5. Zootechnical performances of fish

Settings	T_0	T_1	T_2	T_3	*P*
WG	226.6±21.22[a]	205.93±15.33[a]	211.93±3.71[a]	279.27±17.97[a]	*0.2795*
ADG	0.4±0.025[a]	0.393±0.014[a]	0.378±0.018[a]	0.332±0.034[a]	*0.2453*
SGR	2.58±0.115[a]	2.54±0.064[a]	2.47±0.082[a]	2.25±0.165[a]	*0.2334*
CI	1.20±0.145[a]	1.22±0.08[a]	1.18±0.009[a]	1.41±0.132[a]	*0.4272*
SR	98.33±1.67[a]	95±2.89[a]	98.33±1.67[a]	96.67±1.68[a]	*0.6265*
MR	1.67±1.67[a]	3.33±1.67[a]	0±0.00[a]	0±0.00[a]	*0.2192*
CR	0±0.00[a]	1.67±1.67[a]	1.67±1.67[a]	3.33±1.67[a]	*0.4872*
IP(g)	171.80±3.9[ab]	109.34±8.9[bc]	129.04±3.6[b]	106.91±4.9[c]	*<0.0001*
PEC	1.26±0.01[a]	1.77±0.01[b]	1.56±0.18[c]	1.58±0.14[c]	*<0.0001*

Values are expressed as means ± standard deviation. Values with the same alphabetical letters on the same line are not significantly different at the threshold of α = 0.05. WG: Weight gain; ADG = average daily gain; SGR: specific growth rate; SR: survival rate; MR: mortality rate; CR: cannibalism rate; CI: consumption index; IP: Ingested protein; PEC: Protein efficiency coefficient

3.5 Digestive utilization coefficients

The protein levels obtained in the carcasses as well as the productive protein values are presented in Table 6.

Table 6. Carcass protein level

Treatments	Protein level
T_0	46.75[a]
T_1	37.59[c]
T_2	42.57[ab]
T_3	43.5[ab]
P	<0.0001

Values with the same alphabetical letters on the same line are not significantly different at the threshold of α = 0.05.

Table 7 presents the apparent digestive utilization coefficients of protein (DUCa Proteins) for the different treatments. Protein digestibility varies depending on the treatments. A significant difference between the values obtained from the treatments was observed using the Student t-test. The highest digestibility rate was observed in treatments T_0 and T_2 followed by treatments T_3 and T_1 with low rates.

Table 7. Variation in apparent digestive utilisation coefficients

Treatments	DUCa
T_0	95.12[a]
T_1	88.47[c]
T_2	92.21[b]
T_3	90.37[bc]
P	<0.05

Values with the same alphabetical letters on the same line are not significantly different at the threshold of α = 0.05

4. DISCUSSION

4.1 Physico-chemical parameters of water

The temperature varied between 30.87±0.62 and 31.20±0.63°C and did not show any significant difference. These temperature variations remained within the tolerance ranges of *Clarias gariepinus* (8 to 35°C) recommended by Edéa*et al*. (2019) [8].
Statistical comparisons of the means of dissolved oxygen showed a significant difference between treatments. We noticed that the oxygen level increased with the rate of incorporation of soy okara. This can be explained by water pollution which decreases when the level of fish meal decreases in the food. Fish would pollute the water more with a diet rich in protein.
The pH values fluctuated between 5.42 and 5.58. These values are below those (6 to 8.5) recommended by the Indian Standards Institute [9] or 6.5 to 9.0 according to Hepher and Pruginin[10]. These low water pH values can be explained by the acidity of borehole water in the district of Abomey-Calavi[11]. The statistical comparison of the pH means showed a significant difference between the treatments. We noticed that the average pH decreased with the increase in the incorporation rate of soy okara. This can be explained by the fermentation of the okara which could take place before its use.

4.2 Weight-length relationship in fish

The Student t-test showed that the coefficient b differs significantly from 3 for the four treatments. The growth of *C. gariepinus* fries subjected to these different diets is therefore allometric. The fact that b is < 3 for all treatments means that *C. gariepinus* grew more in length than in weight with these diets containing soy okara. Coulibaly [12] had positive allometric growths for the same species while studying the types of growth within populations of this species in the Sourou River in Burkina-Faso. Overall, the value of “b” varied from 2.5 to 2.65. This range of the value of “b” is consistent with that usually reported and accepted by the literature and which placed this value between 2.50 and 3.50 [13]. This proves that the incorporation of soy okara into the diet of *C. gariepinus* does not modify the growth pattern of this species.

4.3 Fish condition factor K

By comparing the average K values obtained in the different environments, significant differences were observed. We noted an increase in overweight K with the crude protein level of the different diets, which would explain the variations between the K factors.

4.4 Zootechnical and feed utilization parameters

The results obtained showed us that the final average weight, ADG and SGR decreased with increasing soy incorporation rate. This can be explained by the fact that *C. gariepinus* is mainly omnivorous with a carnivorous tendency and therefore uses less soy. However, no significant difference was noted in the various parameters calculated, namely: the average daily gain (ADG), the specific growth rate (SGR), the survival rate (SR), the mortality rate (MR), the cannibalism rate (CR) and the consumption index (CI). These results showed that soy okara does not significantly affect growth, survival and feed utilization parameters. Furthermore, the results showed a significant difference between the protein efficiency coefficients of the different treatments. We can then remember that proteins are more

effective with a low incorporation rate (10%). Wedekind [14], by studying the biological performance of *C. gariepinus* as a function of diet and sex, obtained specific growth rates of 3.7; 3.9; 4.2 and 4.5 by feeding *ad libitum* individuals of *C. gariepinus* having average weights of 5-150g, 5-170g, 5-220g and 5-320g respectively in captivity. The low values obtained for this test can be explained by the size of the individuals which is smaller than that used by Wedekind. Furthermore, the results obtained are better than that (1.7%/day) obtained by Degani *et al*. [15] by studying the effect of different protein levels and temperature on feed utilisation, growth and carcass composition in 3.5-21.5g individuals of *C. gariepinus*.
The survival rates varied between 95 and 98.33% and are consistent with those obtained by Diallo [16] which are 90% and 98.18% respectively for *Clarias gariepinus* fries fed on cottonseed meal and sesame cake.

4.5 Digestive utilization coefficients obtained in fish

Protein digestibility in diets containing soy okara was evaluated in previously described *Clarias gariepinus* individuals, according to the relationship [17]. The apparent DUC of proteins ranged from 88.47 to 95.12%. The protein digestibility of soybean by-products is greater than 90% [18] in common carps and more than 84.06% in Rohu *Labeorohita*[19]. The results obtained for this study are similar to those obtained by these authors with the incorporation of soy in the diet of *Clarias gariepinus* fries. These results are also higher than that (86.8%) obtained by incorporating soya into the carp diet [20]. In addition, they are higher than that (69.5%) obtained in *Leptobarbushoevenii*[21] and that (76.08%) obtained in *Anabas testudineus*[22] by studying the digestibility of soy in these species.
Similar to our results, a protein digestibility of 85% was reported in the catfish *Ictalurus punctatus*[23].
Statistical analyses revealed that the results are significantly different ($p < 0.0001$). The best results are obtained with diets T_0 and T_2 followed by diets T_3 and T_1 respectively. This can easily be explained by the presence of antinutritional factors present in soy [24] which inhibits the digestibility of nutrients. This is also confirmed by the low values obtained in growth. Paradoxically, the T_2 diet gives better results compared to the T_1 diet which nevertheless contains less soy okara. This may be explained by the high protein level in the T_2 diet compared to the T_1 diet and also by the rate at which antinutritional factors might be negligible.

4. CONCLUSION

From this study, it can be deduced that soy okara can be used in the formulation of food for *Clarias gariepinus* fries. It can be incorporated up to 30% without hindering the growth of these fries. The best results were obtained with 20% incorporation, we therefore recommend an incorporation of 20%. Incorporating 20% soy okara into the feed of these fry considerably reduces the cost of the feed and therefore the cost of production since it is a by-product available at a fixed price on the market. In addition, using 20% soy okara could help reduce fish meal and thus contribute to food security since the used fish meal could now be used for other purposes. Furthermore, using soy okara as a substitute for fish meal makes it possible to reduce water pollution in livestock infrastructures and also in the aquatic ecosystems receiving aquaculture effluents.

AUTHORS' CONTRIBUTIONS

'SEGNON Constant Bienvenu' designed the study, performed the statistical analysis, wrote the protocol, andwrote the first draft of the manuscript. 'Arnauld S.M. DJISSOU', 'Diane N.S.

KPOGUE GANGBAZO' and 'André B. ABOH' managed the analyses ofthe study.All authors read and approved the final manuscript.

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