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Original Article

Effect of peanut powder (*Arachis hypogeae* L., 1753) on zootechnic parameters and sex inversion in catfish *Clarias gariepinus*

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Abstract

Benin is currently experiencing an overexploitation of piscatorial resources; this requires the research of endogenous means to increase the biomass of fish produced thanks to fish farming activities. The present study intends to improve the zootechnic performances and inverse the sex in catfish *Clarias gariepinus*. Therefore, 240 larvae obtained from artificial reproduction were used for this study. Three different feed were tested. The control feed (T0) was without peanut powder; contrary, the two experimental feeds were containing the powder at the rates of 10% (T1) and 20% (T2). The best growth of 94.51±27.14 g was recorded with the treatment T2 and 71.32±25.58 g from treatment T1 and finally 54.83±22.19 g from the control group. The sex inversion rate varied from 50% in the control group to 66.13% in lot 1 then 80.13% in lot 2. However, survival rates were low and varied from 26.25% for T2, to 30% in T0 then 42.5% in T1. This study permitted to get better results about the zootechnic parameters and the sex inversion in *Clarias gariepinus* at incorporation rates of 10% and 20% of peanut powder *"Arachis hypogeae*."

Introduction

In Benin, the national fish production is estimated to 110 000 tons in 2012 (Direction des pêches, 2013). Fishing and aquaculture are the most important sources of animal protein. According to FAO (2008), fishing and aquaculture play a vital role in rural, national and global economy. They represent the main source of incomes for 55 million people. World widely, fishing and aquaculture sector produced in 2012 about 128 million tons of fish for consumption (FAO, 2012). Fishing and aquaculture constitute therefore an important subsector in the economy of Benin. They generate many jobs, favour life to population living in wetlands and provide sufficient animal proteins (Gbaguidi and Fiogbé, 1999). Unfortunately, aquatic natural resources are

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confronted to many problems like the overexploitation of fish (Lalèyè et al., 1997). This overexploitation of water resources in Benin is noticeable by the multiplication of sedentary fisheries and the use of prohibited tools despite the existence of regulations (Gbaguidi and Fiogbé, 1999). The demand in fish is therefore getting less satisfaction by fishing activities, though the subsector is contributing efficiently to food security. To overcome this deficit, pisciculture is an inescapable alternative for the intensive fish production. In Benin, apart from some species of Tilapia (*Oreochromis niloticus*, *Tilapia guineensis*) and catfish (*Clarias gariepinus*, *Heterobranchus longifilis*, *Chrysichthys nigrodigitatus*) on which were performed feed tests, it necesary to increase the fish biomass production by other endogenous methods (Otémé et al., 1996; Hechts et al., 1997). Even though several previous studies were carried out on hormonal sex inversion, it is important to notice that the methods used in those studies are difficult to be applied in developing countries since the cost of the hormone used for the inversion remains high (Montchowui, 2009). The research of a replacement product for 17 á methyl testosterone usually used in sex inversion in fish justifies the interest of the present study titled “Effect of peanut powder (*Arachis hypogeae* L., 1753) on zootechnic parameters and sex inversion in catfish *Clarias gariepinus*”. It’s main objective is to evaluate the groundnut powder (*Arachis hypogeae*) on the zootechnic parameters and sex inversion in fish (*Clarias gariepinus*).The specific objectives of this study are:

- to assess the zootechnic parameters of *Clarias gariepinus* fed with different amount of peanut powder (*Arachis hypogeae*),
- to evaluate the rate of sexual inversion by peanut powder in fish *Clarias gariepinus*.

Materials

Two hundred (240) larvae obtained from artificial reproduction and three types of feed were used. One control feed T0 without peanut powder; two experimental feeds containing 10% (T1) and 20% (T2) of peanut powder.

Methods

Technique of production of the larvae

The larvae of *C. gariepinus* used in this study were produced at the Laboratory of hydrobiology and Aquaculture of the Faculty of Agronomic Sciences (University of Abomey-Calavi, Benin). The technique of artificial reproduction of *Clarias gariepinus* which permitted to get the larvae is the following:

The parent fish used for the artificial reproduction were having 50 cm of height and 2 kg of weight and bought from the Directory of the Fishing (Ministry of agriculture, livestock and Fishing, Benin).

Mode of operation

Fishes were first calmed with Phenoxy-Ethanol; they have being injected ovaprim at 0.25 mL/fish in order to accelerate the maturation of the gonads. Then the artificial reproduction was carried out on the females using 17 α methyl testosterone. Larvae were harvested on the 2nd day of reproduction.

Larvae breeding

- Experimental platform and sinks loading

  Larvae were kept in sinks. A total of 240 larvae were distributed in three (03) lots, each lot being constituted of two repetitions of 40 larvae.

- Feeds and feeding of the larvae

  Larvae were fed with Artemia (zooplankton from the class of crustaceans that lives in salty water), five times per day i.e. 8 o’clock; 11 o’clock; 14 hrs; 17 hrs and 20 hrs. The cysts of Artemia, once hatch, constitute an excellent feed for the first feeding of the larvae of *Clarias gariepinus*. Three feeds were tested during this study. The food 0: 0% of peanut powder for three (3) months; food 1: 10% of peanut powder for three (3) months and the food 2: 20% of peanut powder for three (3) months.

Conduction of the larval breeding

Dead fishes were recovered every day. They were examined in order to identify the reasons of their death (cannibalism, acclimatization). Physico-chemical parameters were reported daily.

Fingerlings breeding

- Experimental platform

  After a month, fishes were transferred in bigger tanks which dimensions were 1 m * 1 m * 1 m. They were covered at three quarter (3/4) with covers in bamboos in order to give to fish a shady zone and to limit stress related to human presence. Every tank was provided with water supply pipe and a drainage system in PVC. The level of water was maintained at 0.5 m with a continuous renewal of water.

- Feeds and feeding of fingerlings

  The feeds used at this stade were the same as those used at the larval phase. The fingerlings were fed for two months. The feeding was done three times per day, at 8, 13 and 18 o'clock. The rationing rate was 5% of the total biomass per tank. At this stage, the individual weight of fish per tank was recorded.

- Conduction of the fingerlings breeding

Mortality recording

Dead fishes were recovered every day. They were examined in order to identify the reasons of their death (cannibalism, acclimatization). The physico-chemical parameters were recorded every day.

Data treatment

At the end the experimentation, growth and sex inversion parameters were determined:

Average final weight=Average of individual weights of fingerlings (1)

Average weight gain=Average final weight (AFW) Average initial weight (AIW) (2)

One of the most important parameters was the survival rate because it helps to appreciate usefulness of the feeds used

$$\text{Survival rate} = \frac{\text{final number of fish} * 100}{\text{intitial number of fish}} \quad (3)$$

The specific growth rate SGR gives the speed of growth of the fishes

$$\text{SGR} = \frac{\text{Ln(AFW)} - \text{Ln(AIW)} * 100}{\text{t}} \quad (4)$$

SGR : Specific growth rate

Ln : Naperian Logarithm

t : length within two fishing for control

AFW : Average final weight

AIW : Average initial weight

The consumption index (CI) assesses the effectiveness of the feed used for the fish growth

$$\text{CI} = \frac{\text{Amount of feed served}}{\text{weight gained}} \quad (5)$$

Number of male and female fishes

$$\Delta pm = nfm - nim \quad (6)$$

$$\Delta pf = nff - nif \quad (7)$$

Δpm : variation of male fish

Δpf : variation de female fish

nfm : final number of male

nim : initial number of male

nff : final number of female

nif : initial number of female

Rate of sexual inversion in male (TISm)

$$TISm = \frac{nfm}{ntp} \quad (8)$$

TISm : Rate of sexual inversion in male

nfm : final number of males

ntp : total number fishes

Medium cost of one Kg of fish=cost of one Kg of feed*Qn (quantity)

Statistical analysis

The various parameters recorded during the study were subjected to Statistical Analysis. Thus, physico-chemical parameters (pH, temperature and dissolved oxygen) and the parameters of the use of feed as well as inversion sex parameters were subjected to one way Analyses of Variance (ANOVA 1) with the Software Minitab 14.1. These analysis were followed by multiple comparison tests of Tukey.

Results

Physico-chemical parameters

Temperature

- Temperature in sinks

The average temperature recorded during the larval breeding phase was about 27.70±0.75°C. The temperature evolution in the sinks was similar for the treatments 1 and 2 with a little interval in treatment 1. Extreme values were recorded from treatments 0 and 1 with respective values of 30.6°C and 24.4°C. No significant difference was recorded between the three treatments (Table I) (p>0.05).

TABLE I: Temperature evolution in the sinks.

Parameters	*T0*	*T1*	*T2*
T°C	27.88±1.39[a]	27.67±1.38[a]	27.65±1.36[a]

a indicates that there is no significant difference, p>0.05

- Temperature in tanks

In tanks, the extreme values observed for the temperature were 30.7°C and 24.8°C without significant difference (p> 0.05). Temperature values in the tanks are gathered in Table II.

TABLE II: Temperature evolution in tanks.

Parameters	*T0*	*T1*	*T2*
T°C	27.8±1.67[a]	27.49±1.50[a]	27.48±1.52[a]

a indicates that there is no significant difference, p>0.05

pH

- pH in sinks

The lowest pH was 5.08 and the highest was 6.95 from treatment 0 (Table III).

TABLE III: pH evolution in sinks.

Parameters	*T0*	*T1*	*T2*
pH	5.94±0.33[a]	5.93±0.28[a]	5.88±0.27[a]

a indicates that there is no significant difference, p>0.05

- pH in tanks

The extreme values were 4.25 and 6.79 for treatment 0 without significant difference with the other treatments (p>0.05) (Table IV).

TABLE IV: pH evolution in tanks.

Parameters	*T0*	*T1*	*T2*
pH	5.56±0.77[a]	5.30±0.51[a]	5.25±0.53[a]

a indicates that there is no significant difference, p>0.05

Dissolved oxygen

- Dissolved oxygen in sinks

The dissolved oxygen was similar in all sinks during the larval breeding phase. The extreme values were 6.08 mg/l and 3.06 mg/l for treatments 1 and 2 (Table V) ($p>0.05$).

TABLE V: Evolution of the dissolved oxygen in sinks.

Parameters	*T0*	*T1*	*T2*
O_2	4.37±0.47[a]	4.36±0.63[a]	4.33±0.63[a]

a indicates that there is no significant difference, $p>0.05$

- Dissolved oxygen in tanks

  The table 6 below shows the values of the dissolved oxygen in tanks. The extreme values recorded were 6.36 mg/l and 3.5 mg/l from treatment 0 and 2 without significant difference ($p>0.05$) (Table VI).

TABLE VI : Evolution of the dissolved oxygen in tanks.

Parameters	*T0*	*T1*	*T2*
O_2	4.78±0.72[a]	4.58±0.47[a]	4.60±0.41[a]

a indicates that there is no significant difference, $p>0.05$

Rate of sex inversion

The sex inversion rates were 50%, 66.13% and 80.13% respectively for treatments 0, 1 and 2 with significant difference ($p<0.05$) (Table VII).

TABLE VII: Sex inversion rate.

Treatments	*T0*	*T1*	*T2*
Initial number of fish	80	80	80
Final number of fish	24	34	21
Sex inversion rate (%)	50[a]	66.13[b]	80.13[c]

a, b and c indicate that there is a significant difference at 5% level of significance

Biologic parameters

Survival rate and mortality

Survival rates were low and varied from 26.25% to 42.5% (Table VIII) ($p>0.05$). After breeding larvae for three months, 24, 34 and 21 larvae survived respectively in T0, T1 and T2.

TABLE VIII: Survival rate and mortality.

Treatments	*Initial number*	*Number of dead*	*Survival rate (%)*
T0	80	56	30[a]
T1	80	46	42.5[b]
T2	80	59	26.25[a]

a and b indicate that there is a significant difference at 5% level of significance

Weight evolution of the larvae

The average initial weight registered was 0.00175 g in all treatments. But at the end of the experimentation, the highest values were recorded with the treatment 2 (94.51±27.14 g) followed by treatment 1 (71.32±25.58 g) and finally treatment 0 (54.83±22.19 g) (Table IX).

Evolution of the larvae's size

The size of the larvae increased considerably during their breeding phase. At the end of experimentation, the highest recorded size was 70.01±0.28 mm from the treatment 2 followed by treatment 1 with 60.13±0,9 mm and finally the treatment 0 with 57.1±0.07 mm (Table X) ($p<0.05$).

Consumption index

The consumption index varied from 1.64 to 0.74. The lowest values were recorded with the treatments 0

TABLE IX: Weight evolution of the larvae.

Treatment	*Initial Loading*	*Control fishing 1*	*Control fishing 2*	*Control fishing 3*
T0	$0.00175\pm6.7*10^{-19}$	0.12±0.07	14.94±6.39	54.83±22.19[a]
T1	$0.00175\pm6.7*10^{-19}$	0.18±0.13	15.98±8.34	71.32±25.58[b]
T2	$0.00175\pm6.7*10^{-19}$	0.16±0.09	19.16±8.91	94.51±27.14[c]

a, b and c indicate that there is a significant difference at 5 % level of significance.

TABLE X : Evolution of the larvae's size (mm).

Parameters (CV%)	*Treatment 0*	*Treatment 1*	*Treatment 2*
Loading	$3.8138*10^{-14}\pm6.70*10^{-19}$	$3.8138*10^{-14}\pm6.70*10^{-19}$	$3.8138*10^{-14}\pm6.70*10^{-19}$
Control fishing 1	28.29±0.1	35.77±0.01	40.96±0.04
Control fishing 2	41.89±0.1	46.04±0.06	52.75±0.1
Control fishing 3	57.1±0.07[a]	60.13±0.9[b]	70.01±0.28[c]

a, b and c indicate that there is a significant difference at 5%.

and 2, that besides gave the best growths (Table XI).

Assessment of the rearing cost

The analyses of the rearing cost are displayed in Table XII.

TABLE XI : Consumption index (CI).

Treatment	*Amount of feed served during the test (g)*	*Weight gained (g)*	*CI*
T0	1400	855.73	1.64[a]
T1	1400	1315.9	1.06[b]
T2	1400	1888.2	0.74[c]

a, b and c indicate there is a significant difference at 5%.

TABLE XII : Assessment of the rearing cost.

Treatments	*T0*	*T1*	*T2*
Cost of a Kg of feed (FCFA)	3600	3340	3080
Served feed (g)	1400	1400	1400
Total cost of served feed (FCFA)	5040	4676	4312
CI	1.64	1.06	0.74
Average cost of a Kg of fish (FCFA)	2296	1484	1036

Discussion

The discussion will approach the following parameters: temperature, pH, dissolved oxygen, survival rate, weight gain, consumption index, financial analysis and sex inversion.

Temperature

The difference of values recorded from sinks and tanks is due to the continuous renewal of water in the tanks; this brings a coolness in the tanks. In tropical countries, the temperature values recommended for fish farming range between 24 and 35°C according to the results of Poumogne (1998). Mélard (1999) set the optimum growth of *Oreochromis niloticus* and *Clarias gariepinus* between 26 and 30°C. These values are nearly to those reported by Gandaho (2007) who fed fingerlings of *C. gariepinus* with the leaves of *M. oleifera* under temperatures between 25 and 28°C. The results of the present study are similar to those of Agadjihouèdé et al. (2012) who reported an average of 27.5±0.2°C. The lower difference recorded between the values of Gandaho (2007) and the results of Agadjihouèdé et al. (2012) and those registered during this test could be due to the climatic conditions since the three experimentations were carried out during the same period. These values obtained for the temperature during the present study are in agreement with the optimum recommended for the larvae of *Clarias* according to the aforementioned authors.

Dissolved oxygen

The dissolved oxygen is similar in all sinks during the larvae breeding phase. The difference of values recorded in sinks and tanks is due to the continuous renewal of water in the tanks. Kestemont et al. (1989) and Mélard (1999) reported that a dissolved oxygen higher than 3 mg/l constitutes the optimum for a good growth of *Tilapia* and *Clarias*. However, as shown by Mélard (1999), *C. gariepinus* is provided at the adult stage with a morphological adaptation allowing it to support an extremely low or null level of dissolved oxygen. The values of 3.76 and 4.23 mg/l reported by Gandaho (2007) are lower than those recorded during this experiment. Compared to those of Agadjihouèdé et al. (2012) who reported an average of 6.2±0.1 mg/l, our values are still low. The variation of the dissolved oxygen rate between our data and those of Gandaho (2007) as well as those of Agadjihouèdé et al. (2012) is due to the mode of fish loading. Indeed, Gandaho (2007) used a loading rate of 50 fishes/tanks against 100/tanks for Agadjihouèdé et al. (2012) and 20 fishes/tanks for the current study. These dissolved oxygen values obtained did not influence the growth of the fishes.

Hydrogen potential (pH)

The difference of values recorded from the sinks and tanks is due to the salinity of the water used. The values obtained by Gandaho (2007) ranging between 6.2 and 7.98 were higher than our values. In fact, the differences of values can be explained by the environment of the study areas. These values meet the pH standards of 6.5-9 recommended by Boyld (2001).

Survival rate

The results show that the survival rate varied between 26.25% and 42.5%. These poor rates can be explained by the cannibalism and the aggressiveness feature of *Clarias*. This was described previously by Lazard et al. (1990) during the fingerlings breeding phase. Therefore, the low survival rate is the direct consequence of the cannibalism according to Baras et al. (1999). Furthermore, cannibalism can occur among fingerlings of a same generation, this was observed during this experimentation since the fingerlings were often having torn body and perforated eyes. This situation is at the source of the irregularity between the weight and the size of fingerlings of the same batch. These survival rates were lower than those of Gandaho (2007) and of Agadjihouèdé et al. (2012) who fed *Clarias gariepinus* respectively with Azolla, the leaves of *Moringa* and zooplankton that permitted a survival rate of the larvae of *Clarias gariepinus* up to 97%-98%. Nevertheless, these survival rates are higher than those reported by Atikpa (2002) with Azolla which ranged between 16.67% and 23.33%. This difference of the survival rate is related to the fish loading density in the tanks. Indeed, the leaves of *Moringa* and the zooplankton are richer in nutritive elements compared to the powder of *Arachis hypogeae*. The research of M'po (2004) revealed that the leaves of *Moringa* are rich in water (75%) and also in vitamins B1, B2, B3, C and E as well as in minerals and phosphorus. Besides, Gandaho (2007) revealed that its protein content is of 27.1%. Regarding *Arachis hypogeae*, the proposed nutritive values are 5.4% of water; 26.3% of proteins, 48.4% of fats, 17.6% of carbohydrates, 1.9% of fibres, 2.3% of ashes, 1.15% of minerals and 0.5% of other substances.

Weight growth

The registered initial average weight is 0.00175 g per fish in all treatments. Nevertheless at the end of the experimentation, the highest values were recorded from the treatment 2. The proteins level of 26.3% contained in the seeds of peanuts justifies the excellent growth recorded as the rate of incorporation of peanut powder increases in the feed. The various weight gained recorded are 0.61 g/d; 0.79 g/d and 1.05 g/d for the treatments 0; 1 and 2 respectively. These various weight gains are extensively greater than those recorded by Atikpa (2002) while using Azolla. They are also higher than those of Gandaho (2007) with the larvae of *Clarias gariepinus* that varied between 0.16 g/d and 0.33 g/d. Also, they are greater than the weight gains reported by Agadjihouèdé et al. (2012) with the larvae of *Clarias* fed with soft water zooplankton and that varied between 0.009 g/d and 0.011 g/d. This difference is related to the quality of the nutritive substances used to feed the larvae of *Clarias gariepinus*. However, these values remain lower than those of Montchowui (2001) that is of 3 g/d. The weight growth depends on several factors such as the health status of the fish, the environment in which it is bred but especially the ingested food.

Consumption index

The results showed a difference between the utilisation of the various feed by the larvae of *Clarias gariepinus* during the test. In fact, the consumption indexes are respectively 1.64; 1.06 and 0.74 for the treatments 0; 1 and 2. The low value of the consumption index of treatment 2 is due to the high weight growth that is recorded from this treatment.

This justifies the high quality of the feed used and its good consumption by the fishes. These consumption indexes are higher than the values reported by Imorou Toko et al. (2003) and Dessouassi (2004) with *Clarias gariepinus*. However, these values remain lower than those of Gandaho (2007) in the larvae of *Clarias gariepinus* that oscillated between 1.085 and 1.407. The feed containing peanut powder at the rate of 20% was utilised better by the larvae of *Clarias gariepinus*.

Financial analysis

The analysis of the rearing cost revealed a disproportionate evolution between the rearing cost and the various treatments. Treatment 2 gave lowest production cost which is 1036 FCFA followed by treatment 1 with 1484 FCFA and treatment 0 that showed the highest kilogram production cost of 2296 FCFA. Compared to the control feed, the feed 2 was the most effective and more profitable not only because of its higher economic productivity but also for its sex inversion rate. The lowest production cost is 1036 FCFA with the treatment 2 which is due to the reduced cost of peanut seeds in the market.

Inversion of the sex

The results recorded for the sex inversion were 50% for the control group; 66.13% for the lot 1 and 80.13% for the lot 2. The value of the control lot is in conformity with the literature and present therefore a sex ratio of 1:1. The inversion rates of lots 1 and 2 are distinctly greater than the one of the control group. Used for the first time in order to perform the sex inversion in the larvae of *Clarias gariepinus*, the flour of peanut contains zinc. This oligo-element has the properties of blocking as an anti-aromatase the functioning of oestrogen. In fact, anti-aromatases exercise some anti-oestrogenic effects on the expression of an oestrogeno-regulated gene, the B aromatase, in the glial radial *in vitro* and *in vivo* context. Because of the role of the glial cells in the neurogenesis in fish and the suspected role that plays the œstradiol in this process (Menuet et al., 2005), the observed effects raised the question of the effects of the agonist molecules of Ah and ER receptors in the neurogenesis. The aromatase being expressed in cerebral structures involved in reproduction, it is possible that a change of the aromatase expression can affect the gonads of fish. It is still in the intention to increase the fish biomass (since the male fish weigh more than their fellows female of the same age and exploited in the same conditions) that it was used the 17á Methyl testosterone for the sex inversion in fish. If the results recorded with that synthetic hormone are certainly more conclusive with an inversion rate of 96%, the prohibitive price of the hormone dose (32000 FCFA/ 10 mg) against (1000 FCFA/Kg) of the powder of *Arachis hypogeae* constitutes a constraint for the adoption of this technology by the fish farmers. Moreover, the cultivation areas were very favourable for the peanut whose promotion will not only contribute to the improvement of peanuts farmers' income but also of the fish farmers of Benin.

Conclusion

The highest sex inversion rate is recorded with the treatment at 20% of incorporation of the peanut powder. Furthermore, the treatment at 20% also gave the best weight growth in the catfish *Clarias gariepinus* fed with peanut powder (*Arachis hypogeae*). However, the high mortality rates let to conclude that further experiments should be done to have an optimum concentration which while inducing high sex inversion rate and weight gain, won't lead to a high mortality rate.

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