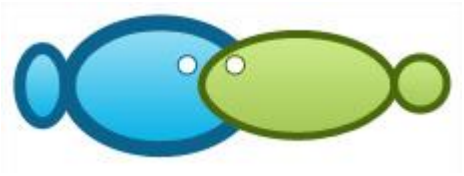

Feed improvement and laserpuncture induction to improve the gynogenetic quality of catfish (*Clarias gariepinus*) fry

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Abstract. An improved feed and laserpuncture induction have been demonstrated to accelerate the gonadal maturation of reproductive catfish (*Clarias gariepinus*) However, it is relatively unclear whether broodstock semen, when irradiated with ultraviolet (UV), will produce the same results in egg fertilization by gynogenesis techniques. Gynogenesis is a parthenogenesis reproduction technique where embryonic development occurs by obtaining genetic material only from females, without male contribution. This study aimed to determine whether the distance of UV radiation on catfish (*Clarias gariepinus*) broodstock semen enhanced by feed improvement and laserpuncture induction will successfully fertilize eggs. The distance between the UV source and semen was divided into 4 treatments namely: control, distance of 10 cm, 15 cm, and 20 cm, with 6 replications. Furthermore, the data collection technique involved a completely randomized design (CRD). The parameters observed included the Hatching Rate (HR), Survival Rate (SR), and Specific Growth Rate Length (SGRL) of the larvae, until the fry were 6 weeks old. The results showed that 20 cm UV radiation distance on semen had a significant effect ($p<0.05$) on the increasing percentage of HR values (63%), SR values (54%), and SGRL (83%) compared to other treatments.

Key Words: hatching rate, specific growth rate length, survival rate, UV radiation.

Introduction. Catfish (*Clarias* sp.) is one of the cultivated commodities with high economic value. The market share for this commodity tends to have an increasing trend. Therefore, the demand for catfish is also increasing. One of the efforts to obtain quality fry involves reproducing only high quality catfish. This requires support by improving the male and female broodstock feeds, thereby improving the quality of eggs, sperm and, subsequently, fry. Quality feeds are necessary to support the survival of embryos and larvae (Izquierdo et al 2001; Çek & Yilmaz 2009)

The presence of probiotics in feed improves the quality, as it increases the digestibility value, growth and survival rate of fish (Crab et al 2012; Iribarren et al 2012; Krishna et al 2015; Chowdhury & Roy 2020; Rodigues et al 2020). In addition, the bacteria present in probiotics also increase the nutritional value of feed by synthesizing vitamins, proteins and essential fatty acids, like amylase, lipase, and proteases (Irianto & Austin 2002; LeBlanc et al 2011; Ray et al 2012; Oktavianawati et al 2016).

In general, genetic engineering can be carried out to improve the quality of fry. This involves the manipulation of chromosomes in the fertilization process, such as gynogenesis. According to Durhan (2004) and Arai (2001), gynogenesis is carried out in two important stages: deactivating the genetic material of male gametes through ultraviolet light (UV) radiation, and restraining the second polar body in meiosis II or restraining the first cell division at mitosis I. This is done by administering a hot shock of

38°C for 3 minutes after fertilization (Volckaert et al 1994; Laczynska et al 2020). When a fertilized egg develops, it produces a diploid female gynogenetic embryo.

UV radiation at wavelengths below 254 nm is proven to be strongly absorbed by certain biological substances, especially nucleic acids, proteins and coenzymes. Furthermore, UV light at a wavelength of 254 nm has been demonstrated to sufficiently damage the function of pyrimidine DNA, which represents the genetic material of male fish sperm (Ijiri & Egami 1980; Valcarcel et al 1994; Saber et al 2017). Although the pyrimidine function of sperm DNA is inactivated by UV radiation, it does not inactivate the ability of sperm to move and fertilize eggs (Zan-Bar et al 2005; Mekkawy et al 2010).

An improved feed and laserpuncture induction have been demonstrated to accelerate the gonadal maturatio of broodstock catfish (*Clarias gariepinus*) (Kusuma et al 2015; Hariani & Kusuma 2019). Kusuma et al (2015) confirm that the use of helium-neon laserpuncture technology on the reproductive acupoint precisely at 2/3 ventral parts of the body through induction for 15 seconds is optimal for the maturation of catfish gonads. Hariani et al (2020) stated that helium-neon low-power laserpuncture technique at the reproductive acupoint of 15 seconds every two weeks is optimal for the maturation of catfish (*Clarias gariepinus*) gonads.

This research focused on improving feed and laserpuncture induction as a reproductive biostimulator to accelerate the maturation male and female catfish gonads. In addition, the semen was irradiated with ultraviolet light (UV) at a certain distance to determine whether it still possesses the ability to fertilize eggs until they hatch.

Material and Method. This research was conducted at the Physiology Laboratory, Faculty of Mathematics and Natural Sciences, PGRI Adi Buana Surabaya University. The data collection method was carried out using a completely randomized design (CRD) with 4 treatments: control, UV radiation distance of 10, 15 and 20 cm, with six replications. Five female African catfish (*Clarias gariepinus*) (900-1500 g and 5 males of 1140-1750 g were used. The fish were obtained from catfish farmers in Pare, Kediri, East Java.

The acclimation of catfish broodstock was carried out separately for 2 weeks in concrete ponds (2x2x0.9 m). Broodstocks were fed with commercial floating pellets (PF-128), with a crude protein content of 38% and probiotics (Probio-7, Tamasindo Veterinary product) with the following composition: *Saccharomyces cerevisiae, Aspergillus oryzae, Lactobacillus acidophilus, Bacillus subtilis, Rhodopseudomonas, Actinomycetes* and *Nitrobacter*, with a density for each bacteria of >1x1011 CFU L^{-1}. Feed was administered twice daily, in the morning and evening, 5% of the body weight, until gonadal maturation.

Eggs and semen collection from the broodstock. Before collecting eggs and semen, the broodstock was treated with laserpuncture at the reproductive acupoint of precisely 2/3 of the ventral part of their body for 15 seconds, with an induction frequency of once every 2 weeks (Hariani et al 2018a). After laserpuncture induction, the catfish were returned to the spawning pond until spawning symptoms were observed. The females showing spawning symptoms were stripped to obtain eggs. The eggs were collected in a dry plastic basin and placed in an icebox at 4°C. Meanwhile, males were dissected to remove the gonads, which were pressed to obtain semen. 1 mL of semen was measured with a pipette and diluted with 9 mL of NaCl physiological/ringer. The semen was then poured into a 1 mm thick petri dish, irradiated with a 15-watt germ lamp (Philips™, Holland) with a wavelength of 260 nm for 2 min, from a UV radiation distance of 0 (no UV radiation) (P0), 10 (W10), 15 (W15), and 20 cm (W20).

Both semen that was irradiated with UV light and not irradiated (control) was mixed with the eggs and stirred evenly using chicken feathers. The fertilized eggs were spread on a tea filter, placed in an incubation container at 28°C for 3 min, then shocked at 38°C for 2 min. This was done to prevent the second polar body's extrusion and ensure that the number of chromosomes remained 2N (diploid). After the temperature shock, the fertilized eggs were incubated in a pond at 28°C until they hatching.

The counting of fertilized and unfertilized eggs was carried out approximately 8 h after the fertilization process in the incubation pond. The fertilized eggs were cloudy white, while unfertilized eggs were clear.

The next step involved calculating the percentage of hatched eggs, or hatching rate (HR), and the survival rate (SR) of catfish larvae until the 5th day of life. Furthermore, the specific growth rate length (SGRL) of gynogenesis results was measured after the 5th day every 2 weeks for 6 weeks using the following equation (Vesal et al 2016):

Hatching Rate (%): HR = a/(a+b) x 100

Survival Rate (%): SR = (Final fish number/Initial fish number) x 100

Specific Growth Rate Length (%/day) = SGRL

SGRL = [(final body length-initial body length)/days of experiment] x100

Where: a - number of eggs hatched; b - number of unhatched eggs.

Data analysis. The data was calculated using SPSS and analyzed using one-way Analysis of Variance (ANOVA) at a significance level of $p<0.05$. The analysis was used to determine the effect of UV radiation distance on the percentage of HR, SR and SGRL of catfish. If a difference between treatments was observed, it was followed by the LSD test. The percentages of HR, SR, and SGRL of larvae before the variance analysis were transformed to arcsin.

Results and Discussion

Hatching rate of catfish eggs. The mean HR values of catfish eggs with and without UV irradiation are presented in Figure 1.

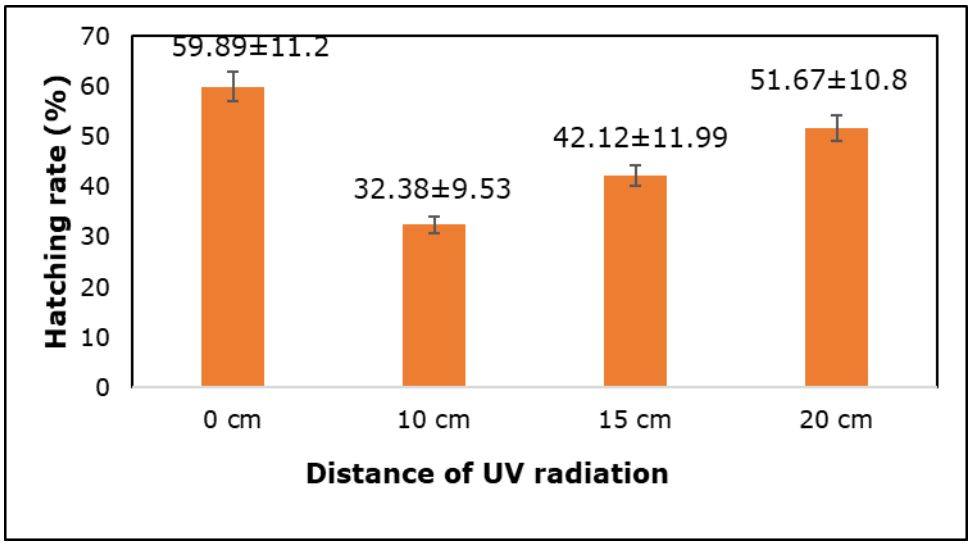

Figure 1. The effect of UV irradiation distance on the hatching rate of catfish (*Clarias gariepinus*) eggs.

The HR of fertilized catfish eggs in P0 was 59.89±11.2%. This was significantly higher ($p<0.05$) than in the other 3 treatments. Furthermore, the HR in W10 was insignificantly lower ($p>0.05$) than that of W15, 42.12±11.99%. The HR in W10 was insignificantly lower ($p>0.05$) than the HR in W20, 51.67±10.8%.

Survival rate of catfish larvae. The average SR of catfish larvae with and without UV irradiation of male catfish semen is presented in Figure 2.

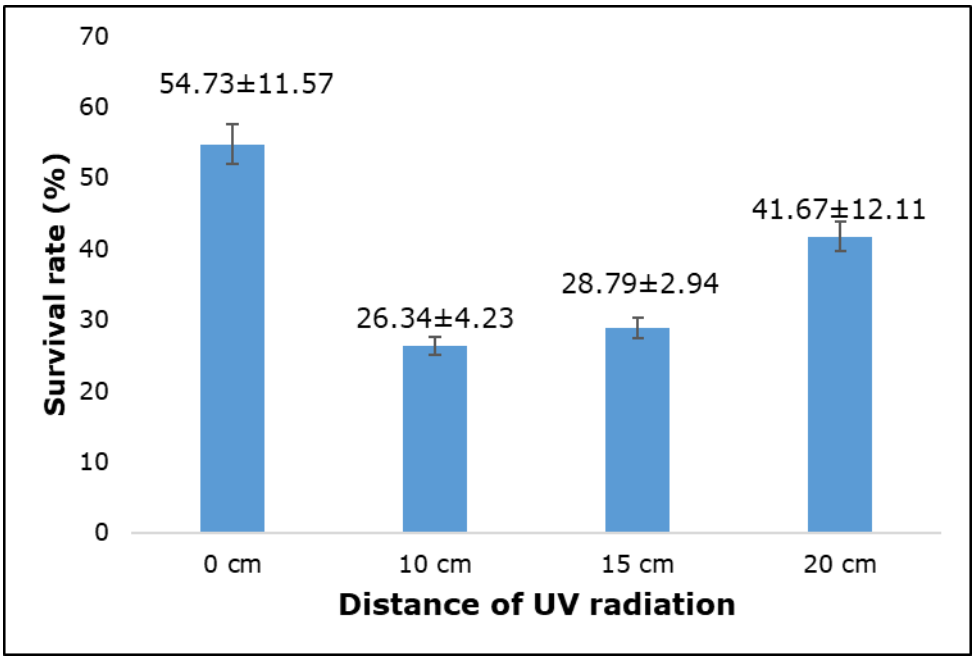

Figure 2. The effect of UV irradiation distance on the survival rate of *Clarias gariepinus* larvae.

The SR of catfish larvae in P0 was 54.73±11.57%. This was significantly higher ($p<0.05$) compared to the SR of the fish in the other treatments. The SR in W10 was 28.79±2.94%, insignificantly lower ($p>0.05$) than the SR in W15. The SR in W15 was also insignificantly lower ($p>0.05$) compared to that of W20, 41.67±12.11%.

Average growth of catfish body weight. The average growth values of catfish fry up to the age of 6 weeks is presented in Figure 3.

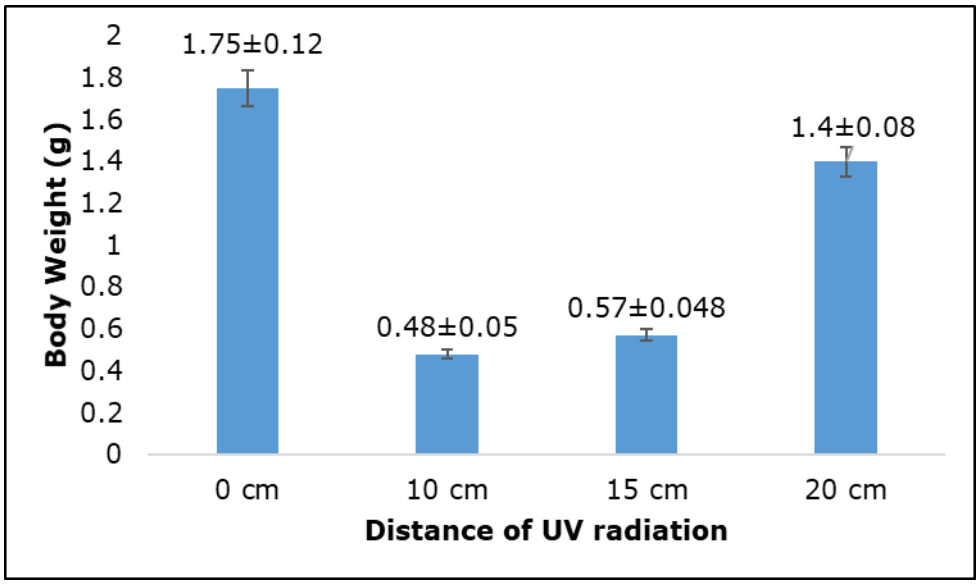

Figure 3. The effect of UV irradiation distance on the growth of average body weight of catfish (*Clarias gariepinus*) fry.

The average growth of body weight up to the age of 6 weeks in P0 was 1.75±0.12 g. This was significantly higher ($p<0.05$) than the average growth value in W10, 0.48±0.05 g. The body weight obtained in W10 was insignificantly lower ($p>0.05$) than the average body weight value W15 (0.57±0.048 g). The body weight obtained in W15 was also insignificantly lower ($p>0.05$) compared to that of W20, 1.4±0.08 g.

Average growth of catfish fry body length. The average growth of catfish body length within 6 weeks of fertilization is presented in Figure 4.

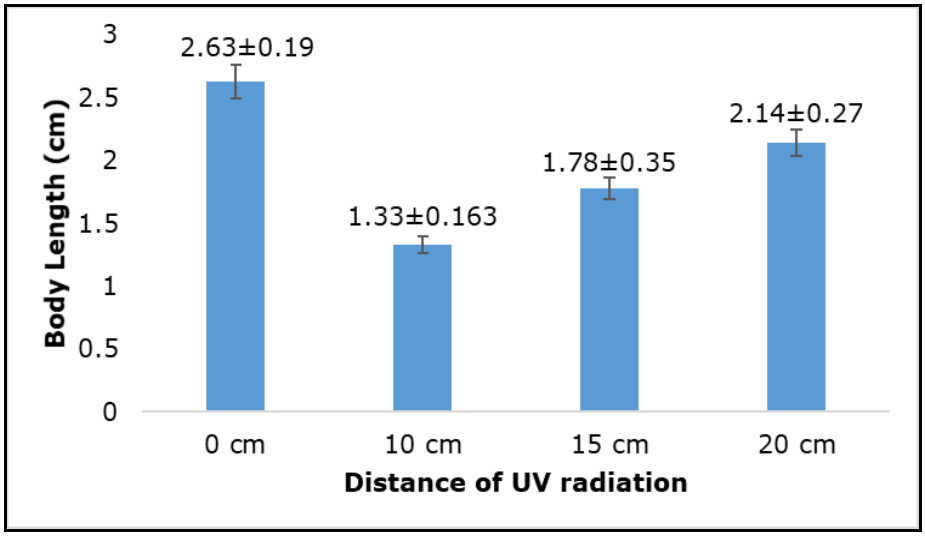

Figure 4. The effect of UV irradiation distance on the average growth of catfish fry (*Clarias gariepinus*) body length.

The average growth of catfish fry length until the 6th week after treatment P0 was 2.63±0.19 cm. This was significantly higher ($p<0.05$) than the average value of catfish fry growth length from the other treatments. In W10, the length was 1.33±0.163 cm, insignificantly lower ($p>0.05$) than in W15 (1.78±0.35 cm). The average growth length in W15 was insignificantly lower ($p>0.05$) than in W20, 2.14±0.27 cm.

Daily specific growth rate length of catfish fry. The daily specific growth rate length (SGRL) of catfish fry of 6 weeks of age is presented in Figure 5.

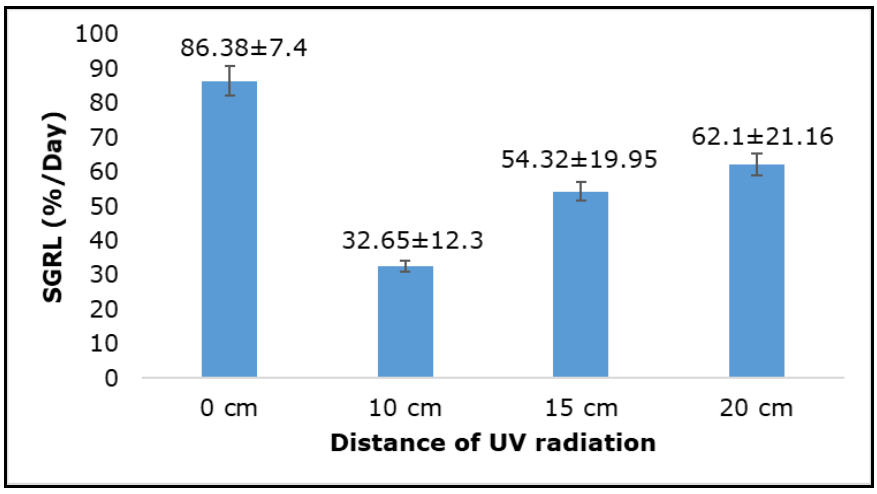

Figure 5. The effect of distance UV irradiation to of growth of average body weight of catfish (*Clarias gariepinus*) fry; SGRL - specific growth rate length.

The SGRL of catfish fry up to 6 weeks of age in P0 was 86.38±7.4%, significantly greater ($p<0.05$) than in the other treatments. W10 had the lowest value. The value in W15 was insignificantly ($p>0.05$) lower than the average value in W20 (62.1±21.16%).

The effect of catfish semen UV radiation distance on hatching rate. HR was influenced by the distance of UV radiation. Bobe (2015) and Baroiller et al (2009) stated that the success of HR is determined by external factors (temperature, dissolved oxygen, pH, sanitation and light intensity), as well as internal factors, including the quality of the eggs and spermatozoa. In this study, the water parameters were maintained in an optimal range.

W20 showed a high percentage of HR compared to W10 and W15, because a closer UV radiation to semen had a negative effect, inhibiting the rate of sperm movement. Therefore, a 20 cm distance of UV irradiation was optimal for destroying the genetic material of spermatozoa without affecting motility. This was evidenced by an increasing enzymatic activity that enabled the movement of spermatozoa to fertilize more eggs. The results of this study were supported by Godwin (2001), who discovered that UV radiation at a wavelength of 254-260 nm determines whether UV rays will penetrate biological materials, especially nucleic acids, proteins and coenzymes. UV radiation at a wavelength of 254-260 nm causes damage to DNA, somatic and genetic cells. In addition, at a wavelength of 254-260 nm, UV light radiation causes chromosomal changes to occur in the cell cycle, especially in the metaphase of meiosis. Cells that divide relatively often have a greater chance of being damaged by UV radiation at a wavelength of 254-260 nm. Furthermore, the success of gynogenesis was also determined by the temperature shock sometime after the egg was fertilized. This temperature shock enables the stimulation of the diploid zygote. Therefore, it should be carried out at the right time to increase the diploidization of gynogenetic seeds, namely during meiosis II and mitosis I (Galbusera et al 2000; Gheyas et al 2001; Tiwary et al 2004).

In addition to the inactivation of semen DNA due to UV radiation distance, its properties to fertilize eggs were no affected, because, in the acrosome part of the spermatozoa, there are hydrolytic enzymes (acrosin and hyaluronidase) that function in egg fertilization. Therefore, there were fewer homozygous larvae in W10, compared to W15 and W20. Due to the proximity of UV radiation to the semen, the temperature shock produced a decrease in enzyme activity, because an increase in temperature will either denature the enzyme or damage the egg cytoplasmic proteins. This enzyme works on the egg membrane, and it consists of pseudoceratine, which works to reduce chorion hardness (Rustidja 2004). Meanwhile, in P0, the HR was significantly different ($p<0.05$) compared to W10 and W20, but the control produced heterozygotic male and female larvae. In P0, W15, and W20 cm, only homozygous female larvae were observed. Another factor that caused a decrease in the HR was the frequency of spawning, which was high and affected the quality of spermatozoa and eggs produced (Bobe 2015).

Effect of UV light exposure distance on the survival rate of catfish larvae. P0 and W10 had medium to high heterozygous catfish larvae, while in W15 and W20, a high number of female homozygous catfish larvae were obtained.

The high and low SR values could have occurred due to the quality of the broodstock. Eggs in W10 had a lower percentage of hatching compared to W15 and W20. Temperature shock also plays an important role in gynogenesis because it holds the second polar body in meiosis II or withstands the division of the first cell at the time of mitosis I. This produces homozygous female individuals (Dunhan 2004).

The results of gynogenesis showed that, as a whole, homozygous females were obtained, whereas the control group produced heterozygous individuals. However, the high level of homozygotism of gynogenesis reduces the ability of larvae to survive.

The effect of UV exposure distance of semen on weight and length growth of catfish fry. The good growth of the weight and length of the fry could have resulted from the use of probiotics in the feed and from the laserpuncture induction. This suggests

that probiotic feed can help increase the growth of catfish fry. Hariani et al (2018b) stated that fish growth is closely related to the availability of protein in feed, because protein is a much-needed nutrient for growth.

The SGRL in 20 cm proved to be optimal. The successful growth of fry weight and length is strongly influenced by the quality of feed administered to the reproductive catfish males and females. Having a better feed quality by using probiotics can increase the vitellin content of eggs and also the quality of the spermatozoa.

The use of probiotics enhanced the feed, and could have improved the digestibility and balance of the microorganism community in the digestive tract (Wang 2007). The ggod growth results are believed to be partially due to the activity of bacteria like the *Bacillus* group and/or *Lactobacillus* sp. from the administered probiotics. Wang (2007) stated that *Lactobacillus* sp. plays a role in balancing the microbe composition of the digestive tract. This increases digestibility by converting carbohydrates into lactic acid, which in turn, lowers pH. Furthermore, this decrease in pH stimulates the production of endogenous enzymes in the digestive tract, increasing nutrient absorption, feed consumption, growth, and the inhibition of pathogenic organisms in the fish body. The *Lactobacillus* sp. bacteria is known to be one of the fermentation microbes present in probiotics. Therefore, when these probiotics are added to the feed, they improve its quality. The digestibility of the feed is better, which in turn increases the weight and length of the catfish fry. According to Irianto & Austin (2002), the work of the first probiotic bacteria involves the suppression of the bacterial population through competition, either by producing antimicrobial compounds, nutritional competition, or the strategic place of attachment on the intestinal wall. The second bacteria play a role in changing the bacterial metabolism by increasing or decreasing enzyme activity and increasing antibody levels in the fish's body.

The finding of these results indicates that to produce quality catfish (*Clarias gariepinus*) fry, it is necessary to prepare male and female brooders before the milt and eggs are collected through proper selection of broodstock, in addition to feed improvement and laserpuncture induction.

Conclusions. Treatment W20 resulted in a HR of 51.67±10.8%, SR of 41.67±12.11%, and SGRL of 62.1 ± 21.165%, higher than in W15 and W10. However, P0 presented better results than W20. The use of probiotics could have improved the growth of fish. More studies are needed on the subject.

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