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SOURSOP (*Annona muricata*) LEAF MEAL FED TO *Clarias gariepinus* PARENT INFLUENCED THE GROWTH AND EARLY DEVELOPMENT OF THE F_1 GONADS

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ABSTRACT

The study was carried out to determine the impact of *Annona muricata* on the growth of the F_1 generations and the gonad development of *C. gariepinus*. 750 hatchlings of average initial weight (0.05g) and length (0.4cm) from the parent that consumed *A. muricata* were stocked in a 1.0x1.0x0.5m^3 fiber ponds in a completely randomized design. The hatchlings were fed 0.2mm coppens as starter (endogenous) feed for two weeks and 0.3 to 0.5 for another four weeks. The hatchlings were weighed weekly to evaluate their growth in weight and length. The fingerlings were further restocked in an outdoor nursery pond 2.0x2.0x1.5m^3 and reared for another three weeks for growth parameters, while the gonad development was evaluated at the tenth week. The result of the growth revealed that the hatchlings had continuous and exponential growth among the treatments; however, the fry that the parent consumed, *A. muricata,* recorded the highest growth and survival rate. While the gonad development of the hatchlings whose parents consumed *A. muricata* started at the eleventh week and the 0% inclusion started at 14th week. These revealed the potential of *A. muricata* in the diet of *C. gariepinus* for the sustainability of the aquaculture industry, the production of quality broodstock, improved growth, and gonad development.

Keywords: *Clarias gariepinus*; weight; length; egg and testis development

INTRODUCTION

Growth is ascertained by an increase in the body weight and length of a fish. It is a coordinated activity of life that correlates with environmental factors such as the quality and quantity of the feed, water temperature, which regulates the amount of feed taken, water pH, dissolved oxygen, and health (Dutta, 1994). However, Parker and Larkin (2011) reported that fish growth can be determined by an increase in size. While From and Rasmussen (1987) exposed that growth is interconnected with its environment, Soderburg (1997) explained that ingested feeds are expressed in the fish biomass. The effectiveness of the conversion is seen in the increase in fish growth. Oluyinka *et al*. (2015) reported the essentiality of the nutrients in the feeds for the growth of the fish. An increase in the weight and length of a fish can be influenced by the nutrients in the feeds, the quality of the water (temperature, pH, and dissolved oxygen) (Volkoff and Rønnestad, (2020), furthermore (Volkoff and Rønnestad, (2020) expressed that the fish are ectoderms, and their physiology are influenced by the water temperature. In addition, the temperature impact their metabolic rate and subsequently influence their energy balance and behavior and the age of the fish.

EGGS AND SPERM QUALITY

Both aquaculture and capture fisheries require mature, viable eggs and sperm for the reproduction of the offspring (Ochokwu *et al*., 2015). Hence, the sustainability of any organism is not achievable when parent stock is not available. As far as aquaculture is concerned, there is a need to know the biology of the species that have been cultured. Eggs are said to be mature and viable when they are fertilized and subsequently hatch, producing young larvae, while the maturity of milt in fish depends on its motility level (Cosson, 2019). It must be able to swim and penetrate an egg for fertilization to occur. Therefore, gamete quality is of utmost importance when the issue of breeding and aquaculture sustainability is concerned (Bozkurt and Secer, 2006). A major problem during the breeding process is when the eggs are not fully mature. Immature eggs will fertilize, and reach 70% hatchability; their survival to fry stage will yield less than 30%, also known as premature fry, and they will look like deformed larvae (Reading *et al*., 2018). Immature eggs are one of the major causes of mortality in the breeding process. Another factor is overfeeding. The fry tend to have a high appetite, and however, feeding them based on their level of feed acceptance and consumption causes mortality. To reduce the mortality caused by overfeeding, 50 fry were randomly selected and weighed, and their weight was used to calculate the amount of feed given based on 5% of their body weight and the number of fry per culture pond (Okomoda *et al*., 2019).

The gonads of a fish are sex organs that procreate gametes (testicle and ovary). Gao *et al*. (2022) explained that gonads are an organ that shows the morphological indication of sexual dimorphism in an animal or fish. The abundance of any organism correlates with the gonads development, maturation, and functionality (Nishimura and Tanaka, 2014). The ovary and testis are the products of gametogenesis. Therefore, reproduction in fish depends on the quality of the sperm and eggs mature fish produce.

African mud catfish (*Clarias gariepinus*) is the most researched fish species in Nigeria because it is abundant, accepted, and has good flesh quality. Its ability to live even in a life-threatening situation made it possible to research it (Okomoda *et al*., 2019). It is of economic value, hardy, has a high reproductive advantage, is nutritious and can effectively be used for aquaculture projects. African mud catfish, *Clarias gariepinus*, are widely cultured; however, the breeding aspects of it have come with challenges like high mortality after yolk absorption, management practices that include water quality parameters (Tiamiyu *et al*., 2018), feeds, and feeding proportions. Little is known about the gonads development in the fish. Hence, the research focused on the effects of *Annona muricata* leaf meal fed to the parent of *C. gariepinus* on the growth and early gonad development of the F_1 (first filial generation).

MATERIALS AND METHOD

Fish and Experimental Design

The research was carried out in the hatchery complex, Department of Fisheries, Modibbo Adama University, Yola, with latitude of 9°13'48"N and a longitude of 12°27'36"E, from January 22nd to July 21st, 2022. The F1 of *C. gariepinus* used for the research was procured from previous breeding exercises, according to Ochokwu *et al*. (2016) and Okomoda *et al*. (2019). In brief, the mature parent of *C. gariepinus* was fed with feeds containing different inclusion levels of *Annona muricata* (D1 0%, D2 1%, D3 2%, D4 3%, and D5 4% inclusion levels) for 56 days (19 January to March, 2022). The mean body weight for both sexes was 550–710 g and the length was 41–46.7 cm. At the end of the feeding trial, the female fish were induced with ovulin at 0.5 ml/kg and male 0.25ml/kg. The latency period lasted for 9 hours at a temperature of 29ºC.The eggs were stripped while the males were euthanized with clove oil before incising the abdomen. A 0.2-ml syringe was used to extract out 0.3 ml of the milt to fertilise 1g of the eggs, while the abdomen was stitched back to heal (7 days) before returning to the pond. The mixture was activated with 5 ml of saline solution for perfect fertilisation and the production of the developed embryo.

GROWTH PARAMETERS

In the first phase (21st March to 2nd May, 2022), 750 hatchlings (F_1) from the parent-fed *Annona muricata* with a mean body weight of 0.04g and a length of 0.7cm were stocked in an indoor flow-through fiber pond (1.0 x 1.0 x 1.0 m^3) in triplicate in a completely randomized design . The 3-day-old larvae were fed *ad libitum* with 0.2mm coppens as a starter feed twice daily, at 7 a.m. and 5 p.m. for 7 days. While 0.3 to 1.0mm were further used based on the weight and length of the fish for 49days. The management practices carried out include general cleanliness of the hatchery, maintaining flow-through operation, and siphoning of uneaten feeds and debris from the pond water. It was observed that the uneaten feeds, unhatched eggs, or debris became a medium for fungi growth. The fungi development and growth began 10 to 14 hours after they remained in the culture pond at water temperature of 27ºC. It is essential to convey that 0.2 mm of coppens feed were used because of its effectiveness, availability, cheapness, and acclaim among the aqua-culturists in Nigeria. The growth in weight and length was monitored weekly.

In the second phase (2nd May to 11th July 2022), at six weeks with body weight 1.22 to 2.09g and length 6.3 to 7.9cm, the fingerlings were transferred to the outdoor nursery ponds 2.0 x 2.0 x 1.5m^3 , they were fed with a 2mm blue crown (42% crude protein). The gonad development evaluation started at the tenth (10th) week, and this was carried out twice: at 11 weeks (prior to 12 weeks) and at 19 week (prior to 20 weeks), and it was done by randomly selecting three juveniles from each treatments. The abdomen was incised while the gonads were snapped using a digital camera to ascertain the development stage.

STATISTICAL ANALYSIS

Data collected for growths in weight and length were presented in a line graph using Excel 2013. While pictographs were used to present the level of gonad developments across the treatments.

RESULTS

The growth in weight (figure 1) of the *Clarias gariepinus* hatchlings (F_1 generation) shows continuous and exponential growth among the treatments. The initial weight (g) ranged from 0.03 in 0% to 0.07g in 4% inclusions. While the final weight (at 8 weeks) ranged from 30.1 g in 0%, to 40.2g in 4% inclusion. The increase in length ranged from 0.3 in 0% to 0.6 in 4% inclusions, while in the final length it increased to 12.9 in 0% and 21.6 in 4% inclusions. The hatchling survival was highest in the 4% inclusions; 94% survived, followed by the 2% inclusions (88%), and the least in the 0% inclusion 53% survived respectively. In the aspect of gonad development in plate 1, the egg development began at 11 weeks among the hatchlings from the parent that consumed *Anona muricata*, and the development was exponential, while the 0% inclusion started at 14 weeks as shown in plate 1.

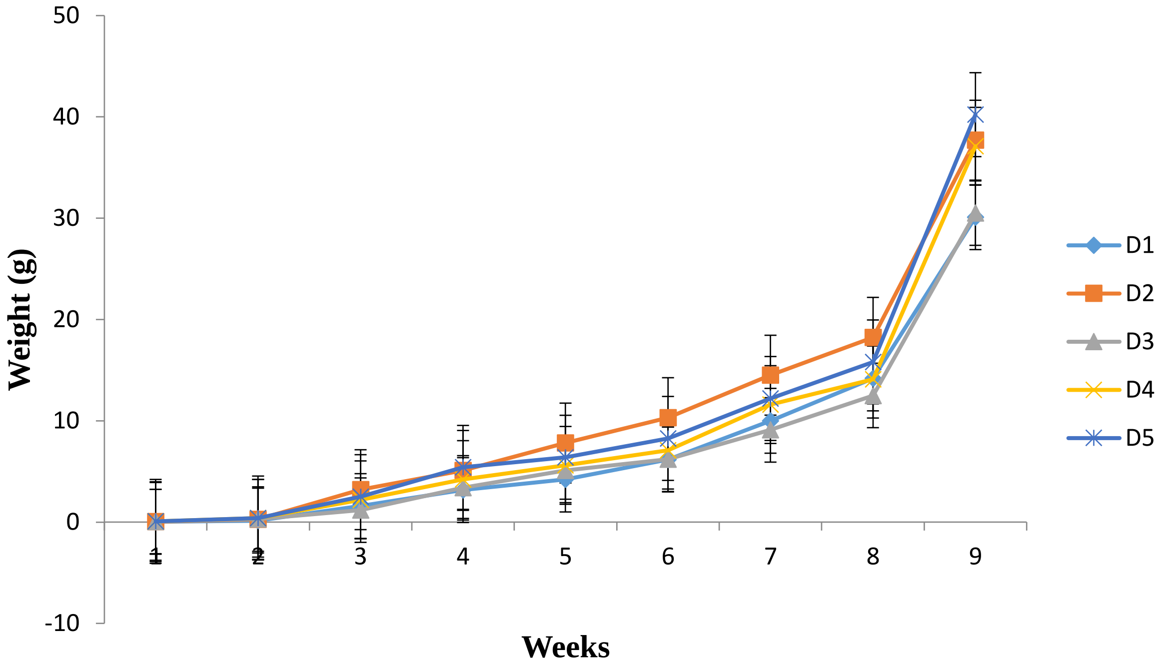

Figure 1. Growth in Weight (g) of the F_1 Generations

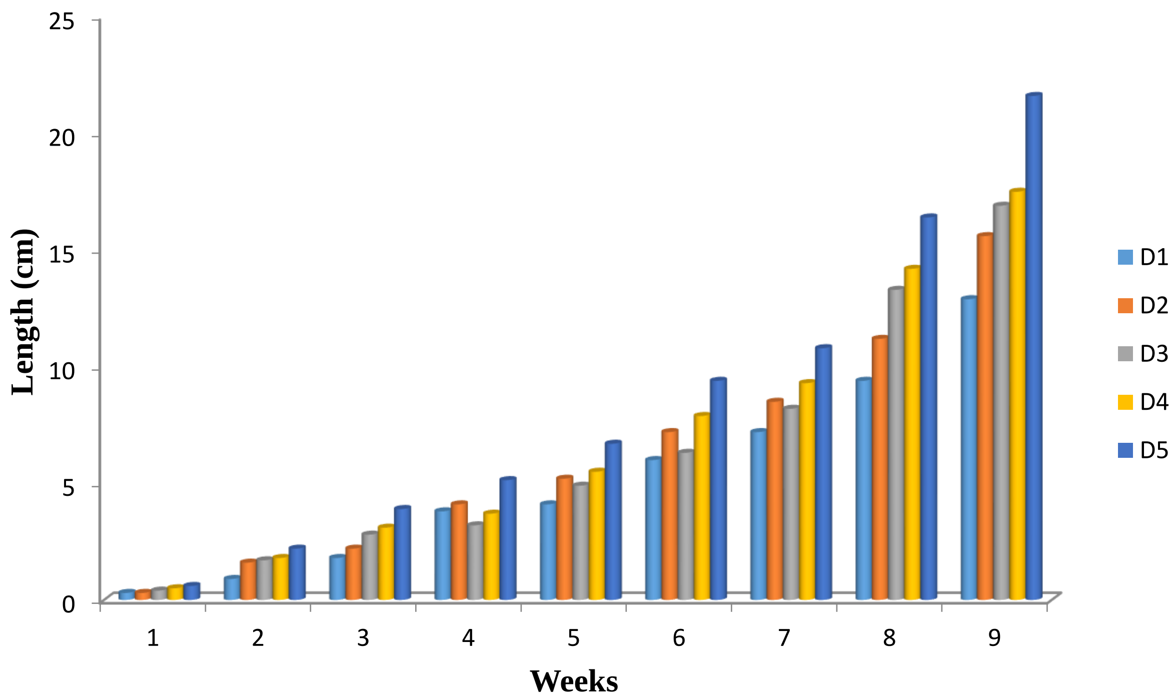

Figure 2. Growth in Length (cm) of the F_1 Generations

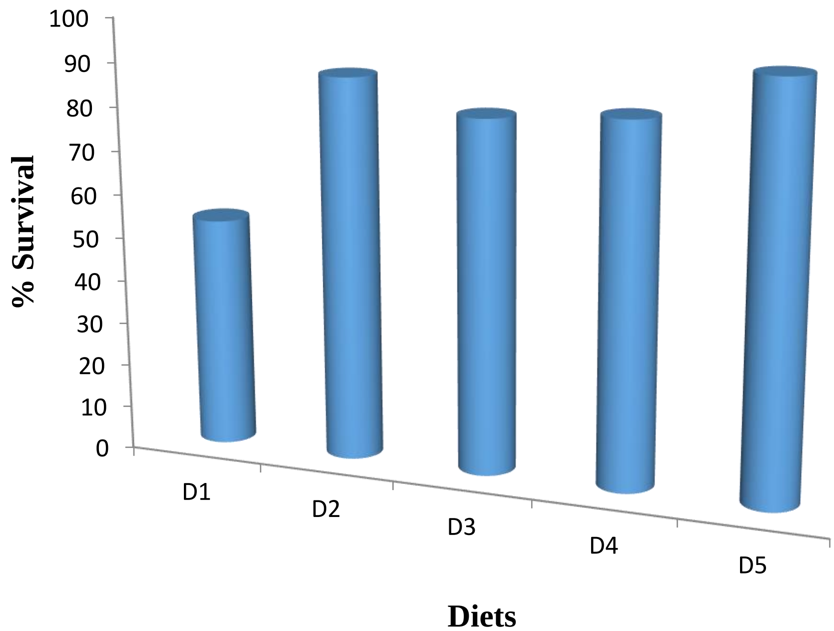

Figure 3. Percentage Survival of the F_1 Generations

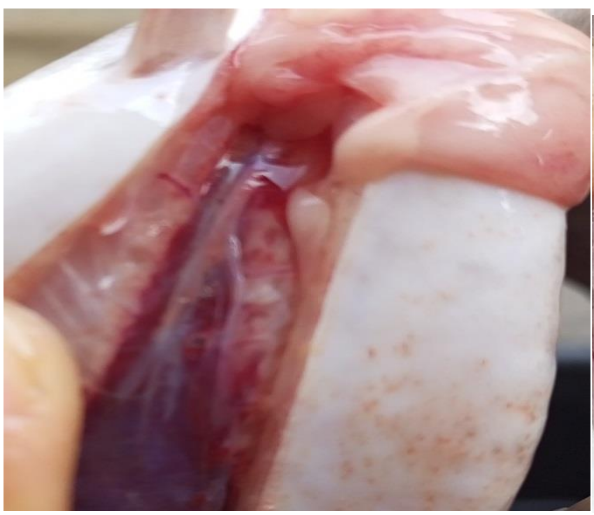

Plate 1a. Egg development of 1% inclusion Of *A. muricata* (11 weeks)

Plate 1b. Testis development of 1% inclusion of *A. muricata* (11 weeks)

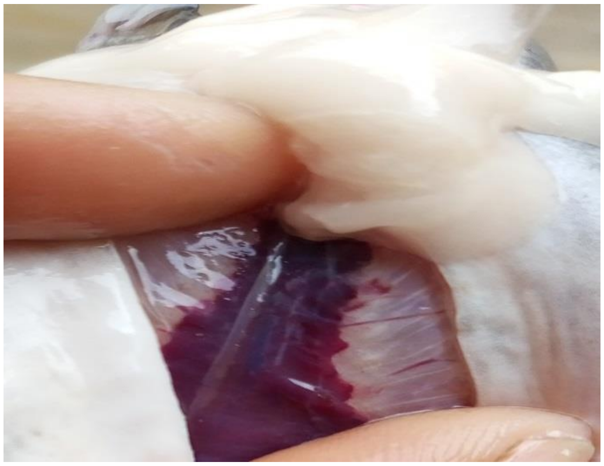

Plate 2a. Egg development of 2% inclusions

Plate 2b. Testis development of 2% inclusions

Plate 3a. Egg development of 3% inclusions

Plate 3b. Testis development of 3% inclusions

Plate 4a. Egg development of 4% inclusions

Plate 4b. Testis development of 4% inclusions

Plate 1a. Egg development of 0% inclusions
19 weeks

Plate 1b. Testis development of 0% inclusions
19 weeks

Plate 2a. Egg development of 1% inclusions
19 weeks

Plate 2b. Testis development of 1% inclusions

Plate 3a. Egg development of 2% inclusions

Plate 3b. Testis development of 2% inclusions

Plate 4a. Egg development of 3% inclusions

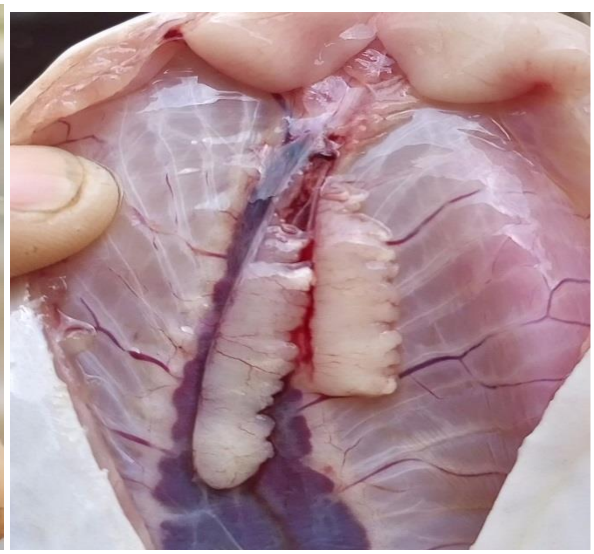

Plate 4b. Testis development of 3% inclusions

Plate 5a. Egg development of 4% inclusions

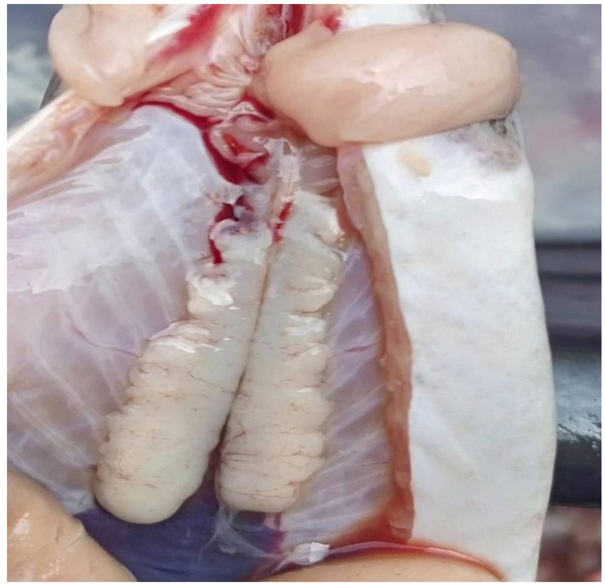

Plate 5b. Testis development of 4% inclusions

DISCUSSION

There was an increase in the weight (g) and length (cm) of the *C. gariepinus* f_1 generation. The 4% inclusion level (Diet 5) performed significantly better than the other fish, followed by 3% (Diet 4), and the least was in 0% inclusion (Diet 1). Sanusi and Bakar (2018) reported that *Anona muricata* is known for its rich nutrient content, such as vitamins, minerals, and bioactive compounds. Some compounds in *Anona muricata* have been studied for their potential immuno-modulatory effects, and the improved immune system of F_1 gives them better resistance to diseases and stress (Kim *et al*., 2016), ultimately leading to increased growth and survival. Another factor is digestibility. *Anona muricata* leaf meal contains some amount of soluble fiber which aids in digestion, leading to better nutrient absorption and utilization (Afzaal *et al*., 2022). Some compounds in *Anona muricata* are linked to metabolic effects, including potential impacts on lipid metabolism. Hence, changes in their metabolic activities could trigger growth (Zubaidi *et al*., 2023). Furthermore, Turner (2009) expounds on the epigenetic (correlation between behavior and environment that triggers a change in the operation of the gene) effect, hence the F1 generation's exposure to *Anona muricata* induced epigenetic changes. Epigenetic modifications can affect gene expression and potentially influence growth (Handy *et al*., 2011). Also interactions with microbiota, the consumption of some plant leaves can influence the gut microbiota of fish. A balanced and beneficial gut microbiota can enhance nutrient absorption and overall health, potentially leading to increased growth (Wang *et al*., 2021). Selective Breeding (Ferosekhan *et al*., 2021), if the F_1 generation consists of fish with naturally higher growth potential and was selectively bred, the improved growth in the F_1 generation might be due to genetic factors (Afzaal *et al*., 2022). Subsequently, F_1 that was fed with *A. muricata* demonstrated a high survival rate when compared with the control (0% inclusion). The parent fish that consumed *Anona muricata* produced high survival progeny. However, inclusion at 4% had the highest survival rate. The high survival recorded can be attributed to the compounds found in *Anona muricata* leaves, such as immunomodulatory properties, which can enhance the immune response of the fish. Kumar *et al*. (2022) report that a stronger immune system can contribute to higher survival rates, thereby reducing the susceptibility to diseases. In addition, Izquierdo *et al*. (2021) stated that the diets consumed by the parent fish can influence the nutrient composition of their gonads (eggs and milt). The nutritional status of the female parent fish can have a significant impact on the survival and growth of the F_1 generation (Riddle and Hu, 2021). Feeding *Anona muricata* to female *Clarias gariepinus* enhanced their health and reproductive success, leading to healthier offspring. The level of maturity of the eggs and milt is crucial to the survival rate of the F_1 Kucharczyk *et al.* (2022). The quality of the eggs produced by female *Clarias gariepi*nus can be influenced by the diet they receive and their ability to absorb nutrients and maximize them in the egg development and maturity (Onyia *et al*., 2015). The general health status of the parent also influences the survival and growth of the progeny (Ferosekhan *et al*., 2021). While Meza-Gutiérrez *et* al. (2022) reported that some bioactive compounds in *Anona muricata* can be transferred from the parent fish to their offspring, potentially providing protective or growth-promoting effects on the F_1 generation.

The gonad development of the F_1 generation revealed that the egg and testis development started at week eleven for the generations in which the parent consumed *A. muricata*, while the control 0% inclusion began at the fourteenth week. The gonads were easily differentiated into male and female after incisions were made on the abdominal part of the fish. Tenugu *et al*. (2021) stated that the gonad development in juvenile fish is a crucial aspect of their reproductive biology and involves the differentiation and maturation of the gonads, which are the organs responsible for producing gametes (sperm and eggs). Understanding the gonadal development of *Clarias gariepinus* juveniles is essential for aquaculture practices, as it will aid the fish farmers in managing breeding programmes and optimizing conditions for reproduction in captivity (Olaleye, 2005). Research in this area contributes to the sustainable management and conservation of the species. The gonadal development in *Clarias gariepinus* juveniles follows a typical pattern seen in many fish species. It involves the development of undifferentiated gonads into either testes or ovaries (Aatsha *et al*., 2022). The process is influenced by various factors, including genetic, nutritional, and environmental cues (Smith *et al*., 2016). In the early stages of gonadal development in fish, Mustapha *et al*. (2021) reported that the gonads are undifferentiated and are referred to as indifferent gonads. During this stage, the fish possess the potential to develop into either males or females. Furthermore, Mustapha *et al*. (2021) repeated that as the juvenile fish grow, they enter the differentiation stage, and the process of differentiation involves the development of either testes or ovaries, depending on genetic and environmental factors. However, from the present research, it was deduced that the gonads can be differentiated at an early stage through the abdominal incision, depending on the genetic, nutritional and health status of the fish. The differentiation is controlled by the interplay of various hormones, including sex steroids (Cavaco *et al*., 1997; Cavaco *et al*., 2001). Once the gonads are attached to the urogenital papillae, the next stage is development (which is increase in size, weight, and length); hence, the differentiated gonads are either testes or ovaries, which grow to maturation. Maturation in fish involves the development of functional gametes (sperm in males and eggs in females) and the ability to reproduce (Rizzo and Bazzoli, 2020). Additionally, factors like nutrition and overall health can impact the growth and development of gonads. *Annona muricata* is known for its nutritional richness, containing vitamins, minerals, and bioactive compounds. It's conceivable that the nutritional profile of the fruit could indirectly influence the overall health and development of fish, including gonads (Meza-Gutiérrez *et al*., 2022). The bioactive compounds present in *Annona muricata* contain acetogenins, which have been studied for their potential health benefits (Mutakin *et al*., 2022). These compounds have physiological effects that, in theory, could influence the fish's reproductive system. Zamudio-Cuevas *et al*. (2014) contributed by expressing that the antioxidant properties of *Annona muricata* will reduce oxidative stress in fish tissues. Oxidative stress can influence various physiological processes, including reproductive development (Pizzino *et al*., 2017). In addition, the anti-nutrients, such as steroids, present in the leaf can boost the gonads maturation.

CONCLUSION

The inclusion of *Annona muricata* in the diet of *Clarias gariepinus* broodstock significantly improved the growth in weight and length of the progeny. The variability was observed when the progeny from the parent that consumed *A. muricata* was compared with the progeny from the parent that did not consume *A. muricata*. The same trend was observed in the survival rate; the progeny (first filial generations) from the parent that consumed *A. muricata* had the best survival when compared with the F_1 from the 0% inclusions. It exposed that *A. muricata*, which is mostly consumed by man has a significant effect on the growth and survival of fish. This is a good report in terms of aquaculture production and sustainability. With this, the fish farmers can limit the mortality level of the progeny when the parents consume *A. muricata*. The significance of *A. muricata* is not limited to only growth; it significantly improved the gonads development and hence brought about early maturation of the eggs and testis. Furthermore, it was further observed that the testes matured before the eggs.

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