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COMPARATIVE STUDY ON THE EFFECTS OF NATURAL (ALOE VERA) AND SYNTHETIC (MTH) INDUCEMENT HORMONES ON SURVIVAL AND MASCULINIZATION OF NILE TILAPIA (*O. Niloticus*).

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ABSTRACT

The comparative effect of natural and synthetic hormones on sex reversal and survival of *O.niloticus* was investigated. Male (N= 5) and female (N=10) broodstocks of average weight 550g and 390g were procured from Bagauda fish farm Kano and transported to the experimental site. Broodstock were acclimatized according to 'sex' while spawning facility (hapas) were placed in each container to facilitate breeding activity and fed commercial feed at 3% body weight for 60 days. After acclimatization, the brooders spawned at a temperature range of 22-29°C, newly hatched fry were collected after 7 days and randomly divided to their treatment containers at sixty (60) fry per treatment. Two experimental feeds containing 17-αMethyl Testosterone Hormone (MTH) and Aloe vera gel (ALV) were prepared. 3g of 17- α Methyl Testosterone Hormone powder was dissolved in 2 litres of 95% ethyl alcohol and mixed thoroughly. 25mls, 50mls and 75mls from the solution was mixed with 250g commercial fry feed, dried and kept inside air tight containers and labelled. Aloe vera leaves were procured, washed thoroughly and the gel was scrapped and blended for a homogenous liquid. Measuring cylinder was used to measure 2.5mls, 3.75mls and 5mls of the Aloe vera gel, then mixed with 250g of commercial diets and allowed todry under room temperature and latter grounded and stored in an air tight container. 60 fry was stocked in triplicates for both treatments in rectangular plastic boxes (20L each) and fed with respective experimental diets and normal commercial diet for the control. Feeding was administered at 5% body weight three times daily for 60 days and water changed weekly. Results obtained revealed that ALV2.5mls had the highest percentage survival (95%) followed by MTH75mls (93.3%) while MTH75mls recorded the highest percentage of fish masculinized (96.42%) followed by ALV2.5mls (96.22%) ($P < 0.05$). Aloe vera gel at 2.5mls/250g of feed inclusion level is ideal for survival and masculinization of *O.niloticus*.

1. Introduction

Aquaculture continues to expand globally as a sustainable alternative to capture fisheries with tilapia species ranking among the most economically significant cultured fish worldwide (FAO, 2023). Fisheries and aquaculture play an important role as sources of food and income, and thus critical in addressing global human food, nutrition insecurity, and economic demands (FAO, 2020). However, global fish production from wild fish stocks has generally stagnated, with the majority of fisheries already fully exploited or over-exploited (FAO, 2020).

Tilapias are the world's second most important farmed finfish (FAO, 2016). Despite the impact of COVID-19, global production of farmed tilapia increased by 3.3 percent in 2020 to reach 6 million tons for the first time (Abu-Elala *et al*., 2020). The ability to produce tilapia in a variety of aquatic environments, selective breeding, and the potential to replace marine fish products have all contributed to the global expansion of tilapia production (Abu-Elala *et al*., 2020). Due to these characteristics, the species is able to adapt quickly to new environments, displaying a diverse range of biological responses to various environmental conditions both in culture and in nature (Schofield *et al*., 2011;

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Grammer *et al*., 2012; Nouman *et al.*, 2021). The Nile tilapia (*Oreochromis niloticus*), in particular, has gained prominence due to its fast growth, tolerance to varying environmental conditions, and high consumer acceptance (El-Sayed, 2021). However, mixed-sex populations often result in uncontrolled reproduction, overpopulation, and stunted growth, which ultimately reduce yield and profitability (Gupta & Acosta, 2020). Consequently, the production of all-male populations through hormonal sex reversal has become a standard practice in commercial hatcheries.

Hormonal sex reversal is the most efficient and is a widely used technique that allows for the mass production of all-male tilapia in both small and largescale production systems (Jensi *et al*., 2016). The synthetic androgen 17α-methyltestosterone (MT) has been extensively used for sex reversal due to its high efficiency in producing all-male progeny and promoting growth uniformity (Pandit *et al*., 2022). Despite its effectiveness, MTH application has raised ecological and public health concerns, including endocrine disruption in non-target organisms and potential residual accumulation in aquatic environments (Yun *et al.*, 2021). Moreover, consumer preference is gradually shifting towards eco-friendly aquaculture practices that limit chemical and hormonal use (Oladimeji *et al*., 2023). This paradigm shift has led to the exploration of natural and plant-based bioactive compounds as safer alternatives to synthetic androgens. Among potential natural alternatives, Aloe vera has attracted attention due to its rich composition of phytosterols, saponins and phytohormones that exhibit androgenic and anabolic effects (Femi-Adepoju *et al.*, 2020). Previous studies have demonstrated the potential of Aloe vera extracts in enhancing growth, improving immune response and stimulating gonadal development in fish (Anitha *et al.*, 2021, Mousa & Khattab, 2022). However, reports on its effectiveness as a masculinizing agent remain limited and inconsistent, particularly in *O. niloticus*. Furthermore, there is limited comparative data between Aloe vera and 17α-methyltestosterone in terms of their influence on growth performance, survival and sex differentiation in tilapia fry.

Furthermore, the information on the use of *Aloe vera* gel (AVG) meal to reverse sex of fish will be able to provide treatment procedures for tilapia precocious breeding and further assist fish farmers to enhance their economic returns by farming profitably with tilapia as well as reduce or eliminate the use of unsustainable synthetic hormones (Reverter *et al*., 2014). This study therefore sought to evaluate the comparative effects of Aloe vera extract and 17α-methyltestosterone on survival, growth performance, and masculinization efficiency in *O. niloticus* fry cultured in a hatchery system over a 60-day period.

2. Materials and Methods

2.1 Study Site and Acclimatization of Brood Stock

The study was conducted in fisheries and Aquaculture unit, National Agricultural Extension Research and Liaison services (NAERLS), Ahmadu Bello University Zaria, Nigeria. Fifteen (15) brood stock comprising five males and ten females with approximate weight of 550g and 390g respectively were procured from Bagauda fish farm Kano and transported in polythene jars between 06:00hrs and 09:00hrs to the experimental site. Brood stock were acclimatized in 1000L containers (3) and filled with 600L water level according to sex while spawning facility (hapas) were placed in each container to facilitate breeding activity (Ghosal *et al*., 2015). The brooders were fed Commercial feed (0.1mm size) at 3- 5% body weight according to (Jegede, 2011) for 30 days where breeding activities were observed. Water temperature and pH were monitored using multi parameter HANNA instrument (model H198129) while Winkler's Titrimetric method (El-Sayed, 2016) was used for Dissolved Oxygen (D. O).

2.2 Hatching of Fry

After acclimatization, the brooders spawned at a temperature range of 22-29°C in their respective containers, eggs were laid by females and fertilized by males. Incubation and brooding occurred within the females afterward. After 7 days, the newly hatched fry were collected by flushing them out from the females' brood stocks mouth in a separate container and randomly divided to their treatments containers of twenty (20) litres at sixty (60) fry per treatment for the experiment (Olaniyi *et al.*, 2020).

2.3 Experimental Feed Preparation

Experimental feed containing 17-αMethyl Testosterone Hormone (MTH) (Treatment A)

3g of synthetic 17- α Methyl Testosterone Hormone powder was dissolved in 2 litres of 95% ethyl alcohol. The mixture was thoroughly mixed to get a clear stock solution. (Fuentes-Silva *et al*., 2013). 25mls, 50mls and 75mls from the stock solution was sprayed using a sprayer and each mixed with 250g commercial fry feed (0.1mm diet) for even distribution and spread on clean dry surface at room temperature and allowed the alcohol to evaporate for three (3) days, after which they were kept inside air tight containers and labelled (Fuentes-Silva *et al*., 2013).

Experimental feed containing Aloe vera gel (ALV) (Treatment B)

Aloe vera leaves (i.e. *Aloe barbadensis*) were obtained from NAERLS garden, ABU Zaria and identified in Herbarium lab of department of Botany. The leaves were washed thoroughly under running tap water. With the aid of

a clean sharp knife, the gel was scrapped from the A. vera leaves and blended for a homogenous liquid (Prasad,2013). Measuring cylinder was used to measure 2.5mls, 3.75mls and 5mls of the Aloe vera gel which were then mixed with 250g of 0.1mm commercial diets respectively and allowed to dry on a clean surface under room temperature and latter grounded and sieved with 0.1mm diameter sieve and stored in an air tight container, labelled accordingly and kept in a cool environment (Prasad, 2013).

2.4 Experimental Set-Up:

The experimental set-up was designed in two phases.

Phase one*:*

Phase one was set up immediately after hatching process. The fry was divided into two experimental groups i.e. Group A; Methyl Testosterone diet (MTH) and Group B; Aloe vera diet (ALV) with a control. Each of the groups was subdivided into three and triplicated following their experimental feed concentrations of 25 (MTH1), 50(MTH2) and 75mls (MTH3) (Group A) and 2.5(ALV1), 3.75(ALV2), 5mls (ALV3) (Group B). 60 fry was stocked in triplicates for both treatments in rectangular plastic storage boxes of 20L each and fed with respective experimental diets and normal commercial diet for the control. Feeding was administered at 5% body weight at 08:00hrs, 14:00hrs and 18:00hrs (Bolorunduro, 2002) for 60 days and water changed weekly.

Phase two;

Phase two was conducted with commercial diet without treatments for a period of four (4) months with water quality growth parameters in check following standard methods.

2.5 Determination of survival rate of Tilapia

Percentage survival was determined according to (Yisa *et al*., 2013) as cumulative survival divided by total number of fish stocked multiply by hundred.

$$\% \text{ survival} = \text{cumulative survival} / \text{Total Number of fish stocked} \times 100 \quad (1)$$

2.6 Determination of Percentage Masculinization of Tilapia

Percentage masculinization was determined according to (Turan and Cek 2007) as Number of male fish divided by total number of fish stocked multiply by hundred.

$$\% \text{ Masculinization} = \text{Number of Male fish} / \text{Total Number of Fish stocked} \times 100 \quad (2)$$

2.7 Data analysis

Data collected were summarized using descriptive statistics, bar chat was used in presenting data collected on survival and masculinization of *Oreochromis niloticus*. Microsoft excel 2016 version was also used in analyzing data. Analysis of variance was used to compare treatment mean, were significance was observed, Duncan's multiple range test was employed to separate the means. All data was tested at 5% level of significance.

3. Results

3.1 Mean Percentage Survivability of Masculinized *O. niloticus*

Masculinized *O. niloticus* has revealed in the study (Figure 1) indicated that treatment bearing MTH1 (25mls) group had the lowest percentage survivability of (85.0 %). Control, treatment ALV2 (3.75mls) and treatment MTH2 (50mls) had equal percentage of survivability of (88.3%) each, followed by treatment ALV3 (5.0mls) and treatment MTH3 (75mls) with 90.0% and 93.3% respectively. Treatment ALV1 (2.5mls) group had the highest percentage survivability of 95% with significant difference over other treatments ($P < 0.05$).

3.2 Percentage Masculinization of Experimental Fish Fed Different Treatment Diets

Percentage masculinization of *O. niloticus* (Figure 1) reveals that Treatment with MTH3 (75mls), recorded the highest percentage of fish masculinized (i.e. 96.42%) and were significantly higher ($P < 0.05$) than treatments MTH2 (50mls), treatment ALV3 (5.0mls), treatment ALV2 (3.75mls), treatment MTH1 (25mls), and treatment ALV1 (2.5mls) which recorded percentage masculinization of (96.22%), (94.44%,) (90.56%,) (88.23%) and (70.17%) respectively. Control had 66.03 % males, indicating significant difference ($P < 0.05$) in percentage masculinization with respect to the five treatments (ALV1, ALV2, ALV3, MTH1 and MTH2).

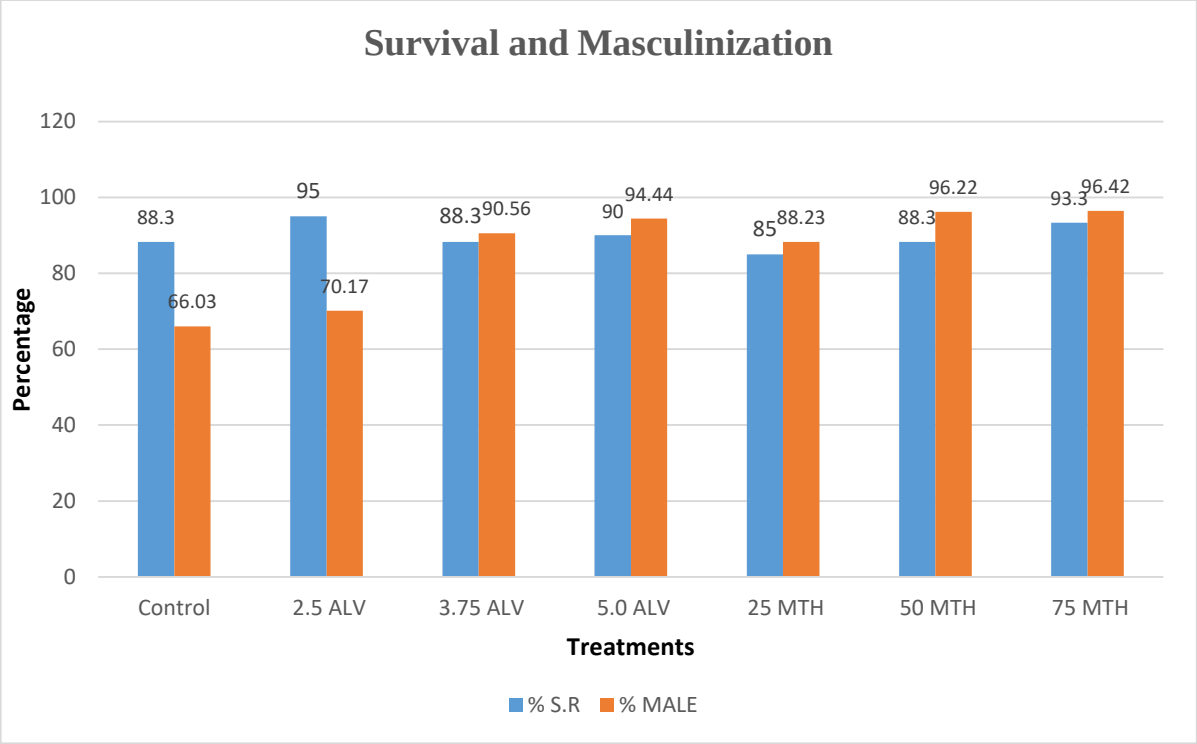

Figure 1: Percentage survival and masculinization of the experimental fish

Key: ALV =*Aloe vera*, **MTH** = Methyltestosterone, **S.R**=Survival rate, **Male**=Masculinization

4. Discussion

The effect of herbal extracts on sex reversal in tilapias was reported in the early 90s (Pelissero & Sumpter 1992). In the present study, herbal extract (Aloe vera) has proven high potency in sex reversal (70.17%) of Nile tilapia with high survival rate (95%) at lower inclusion levels (2.5 mls/g). However, increased inclusion levels (5.0 mls/g) resulted in higher potency (94.44%) but lower survival rates (90.0%) This is in accordance with the findings of (Phelps & Popma, 2020); Pandit *et al.*, 2022) who in their separate studies reported that Aloe vera extract have the potentials to be used as sex reversal agent in Nile tilapia with higher survival rate at lower inclusion level. Plant extracts can be toxic to animals to the point of causing mortality, especially at high doses (Anitha *et al.*, 2021). In the case of A. vera, contradictions on its toxicity exist. Thu *et al*. (2013) reported that A. vera extract had minimal toxic effects on brine shrimp Artemia spp. and recommended that it could be safely used as an antimicrobial agent. Conversely, Taiwo *et al.* (2005) revealed that a dietary A. vera inclusion level of 50 mg/L generated adverse effects in juvenile Nile tilapia, including tissue necrosis, hypoxia and gill, heart, liver and kidney damage. Similarly, (Gabriel *et al.* 2015) reported that a dietary A. vera inclusion level as high as 4.0% per kilogram of feed resulted in vulnerable, highly stressed and anemic juveniles that displayed poor defense mechanisms against physiological stress and energy metabolism (Gabriel *et al.* 2015). This may explain the low survival rate observed in high inclusion levels (5.0 mls/g) of A. vera diet in this study. In Conclusion, A. vera could be used at 3.75mls/250g of feeds maintains high % masculinity (90.56) and high survival (88.83) in Nile tilapia.

This study demonstrates that Aloe vera extract can serve as a potential natural alternative to 17α-methyltestosterone in the masculinization of *O. niloticus* fry. The result suggests that Aloe vera not only enhances growth performance but also confers immunological benefits that improve survival. Given the environmental and safety challenges associated with synthetic hormones, Aloe vera presents an eco-friendly, cost-effective and locally available alternative suitable for sustainable aquaculture.

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