https://doi.org/10.46344/JBINO.2022.v11i01.09

EVALUATION OF HONEY ON THE SEX REVERSAL AND GROWTH PERFORMANCE OF *OREOCHROMIS NILOTICUS*

Onyia,L.U[1].; Sylvanus, N[1]., Onyia. E.C[2]., Isaac, M.K. [1]. and Jibrin, M.T[1].

[1]Department of Fisheries, [2]Department of Zoology, Modibbo Adama University, Yola.

Email : luconyia@gmail.com

ABSTRACT

The research was carried out to examine the effect of different volumes of honey on the growth and percentage male Tilapia. Three hundred fry each weighed 0.066g and 1.75cm were used for the experiment. Different honey concentrations 0 .0,1.0, l..5,2.0 and 2.5, as treatments 1, 2, 3, 4 and 5, respectively per litre of water. The fry were immersed for 24 hours in a glass aquarium (40x40x40cm). Each treatment was replicated three times in complete randomized design (CRD) manner. The fry was put in 2 litres of water that has been blended with honey in accordance with the concentration of each treatment. Twenty (20) fry were immersed in honey solution and left for 24 hours. The result showed that D4 had the highest growth in weight and length(1.74g and 3.62 ± 1.42) and the least was in D1(1.14g and 2.67 ± 0.65) after 5 weeks. D2 and D3 were (1.34 and2.78± 0.80; 1.54g and 3.62 ± 1.42)respectively. D4 had the best Mean daily weight gain, Relative Growth Rate and Specific Growth Rate when compared to the other treatments. There was significant different(P≤0.05) in the growth of the different treatments. Fry were randomly collected from the different treatments, using Squash method, the males and female fry were identified under the microscope(x40). D_4 (40% of honey mixed with 2 litres of water) had the highest percentage males(90%), followed by D3(80%) and D2(69%). D1(Control) had the least percent males(33%). D5 was 50% males. From the result, it could be concluded that 40% honey in 2litres of water could be used to generate high percentage of males that will grow faster than the females.

KEYWORDS: Honey, sex reversal, growth squash method, *Oreochromis niloticus*

INTRODUCTION

Oreochromis niloticus is ideal for culture dues to its high growth rates adaptability to a wide range of environmental conditions, ability to grow and reproduce in captivity ability to feed at low tropics level (Abdel –fattah,2002). *Oreochromis niloticus* mature early and can be easily bred in captivity all the year round. They attain sexual maturity in 4 to 6 months while still being smaller than marketable size. They also spawn frequently (females every two to four weeks during the spawning season). Therefore, culture areas can become saturated with vast numbers of smaller and uneven sized fish, which are often unwanted by fish producers or hatchery operators (Pechsiri and Yakupitiyage, 2005, Megbowon and Mojekwu,2013). Females grow slower in this species. Males, and especially females, divert energy which could be utilized for somatic growth, into gamete production and behavioral interactions. In addition, competition with recruits in confined environments further suppresses the growth of stocked fish and can result in 30-50% of harvested biomass consisting of largely unmarketable recruits (Mair *et al*., 1995). Therefore, in semi-intensive or intensive aquaculture, male tilapia is preferred for culture and harvest to reduce the possibility of unwanted reproduction and stunting in grow-out ponds, to obtain fast growing, more uniform sized fish and to gain more profit(Megbowon and Mojekwu,2013).

Despite promising expectations in *Oreochromis niloticus* aquaculture, there are a series of factors that are considered to be detrimental in the expansion of production. Female organism of tilapia species has a high fecundity, generally reproducing at a small size and exhibiting stunted somatic growth at higher densities, while male tilapias exhibit faster growth rates and are often the preferred gender for monosex aquaculture(Chakraborty *et al*. 2011). Monosex population of male tilapia is produced by treating spawn with a synthetic male hormone 17a-methyl testosterone either through immersion(Firmansyah *et al*., 2016 and Chaves-Pozo *et al*., 2018), addition in fish feed(Soelistyowati *et al.,* 2010) and injection(Ahlina *et al,*. 2015). However, the increased use of synthetic steroid hormones to produce monosex populations of *Oreochromis niloticus* for intensive productive systems may lead to environmental and public health concerns(Sudrajat and Sarida 2006). Moreso, the synthetic hormone is expensive and could be beyond the reach of fish farmers(Damayanti *et al*., 2013; Asaduzzaman *et al*., 2019). Therefore, alternative methods and new, safe chemicals to produce monosex tilapia populations should be considered, such as the use of honey. It is known that natural ingredient that has the same role as synthetic steroid hormones for masculization is honey and safer as well as environmentally friendly(Pattiasina *et al.,* 2021, Kautsari *et al*.,2015, Contreras-Sanchez *et al*.,2001) The objectives of this study are to determine the effects of immersion in honey on sex reversal, on the growth performance of *Oreochromis niloticus,* determine the survival rate of *Oreochromis niloticus* after sex reversal and estimate the ratio of male and female *Oreochromis niloticus*.

Materials and Methods

The study was conducted in the fish hatchery of Department of Fisheries, Teaching and Research farm, Modibbo Adama University Yola, Adamawa State, Nigeria. Adamawa State is in North Eastern part of Nigeria; it is one of the largest states in Nigeria and occupies land size of about 36,917 square kilometres. It is bordered by the states of Borno to the northwest, Gombe to the west and Taraba to the southwest. It is located on latitude 9.20 – 9.33^{0}N, longitude 12.30 – 12.50^{0}E and an altitude of 185.9m. It has an average annual rain fall of about 759mm with maximum temperature of 39.7^{0}C.

Source of brood stock

Male and female *broodstock* of *Oreochromis niloticus* was obtained from Department of Fisheries, Teaching and Research farm, Modibbo Adama University of Technology Yola, Adamawa State, Nigeria. The selected brood stock were acclimatized for 1 week and fed commercial fish feed. After which, they were stock in the ratio 2 females: 1 male and allowed be breed.

Preparation of Honey Solution

Honey used for this experiment were obtained from Mayo Belwa, Adamawa State. Different honey concentrations:0, 1.0, 1.5, 2.0, 2.5% of honey per/litre and will be constituted into 2litres of water. The honey and water were thoroughly mixed by stirring until a homogenous solution will be obtained.

Experimental design

Three hundred fry were used for the experiment. Different honey concentrations (1.0, l. .5,2.0, 2.5 and 0 .0) as a control, as treatments 1,2, 3,4 and 5, respectively per litre of water were used for the immersion for 24 hours in a glass aquarium (40x40x40cm). Each treatment was replicated three times in complete randomized design (CRD) manner. The fry was put in 2 litres of water that has been blended with honey in accordance with the concentration of each treatment. Twenty (20) fry were immersed in honey solution and left for 24 hours.

Procedure of the Applications of squash technique in sex determination in fish

- Fish are sacrificed and the gonads are excised using fine forceps. (a magnifier may be required). Collected gonads are mounted on a glass slide and few drops of aceto-carmine stain are added. Gonads are lightly squashed with a cover slip
- The gonad mounts were examined under a compound microscope using magnification of 25 to 50. The male gonad is composed of fine granular like structure of spermatogonia and the female is characterized with the structure of circular oogonia
- Often, females are easily identified compared to males. The number of tested fish (gonads) is related to statistical requirements; a random sample of 30 specimens were used(Guerrero and Shelton, 1974)

- **Growth Performance of Fry**
- The hatchlings were fed powdered commercial feed at 10% percent body weight, three times daily.
- The following formulae were used for the growth analysis according to Adebayo and Popoola (2008):
- Mean of the daily weight gain = $\frac{final\ weight - initial\ weight}{culture\ period\ in\ days}$

- Relative growth rate (RGR) = $\text{Log}_e \frac{(final\ weight) - Log_e\ (initial\ weight)}{culture\ days}$
- Specific growth rate (SGR) = $\text{Log}_e \frac{(final\ weight) - log_e (initial\ weight)}{culture\ days} x\ 100$
- Survival rate = $\frac{final\ number\ of\ fish}{initial\ number\ of\ fish} x\ 100$
- **Histological studies**
- The gonads of one male and female were randomly collect from each treatment. The gonads were preserved in 10% percent buffered formalin prior histological process according Carson *et al* 2009.
- **Statistical Analysis**
- Data will be subject to one-way analysis of variance (ANOVA)and when ANOVA indicated that there was statistical difference between stockings densities means, will be used to compared these means to determine whether significantly difference existed between the different treatments and parameters tested at 5percent level of significance.
-

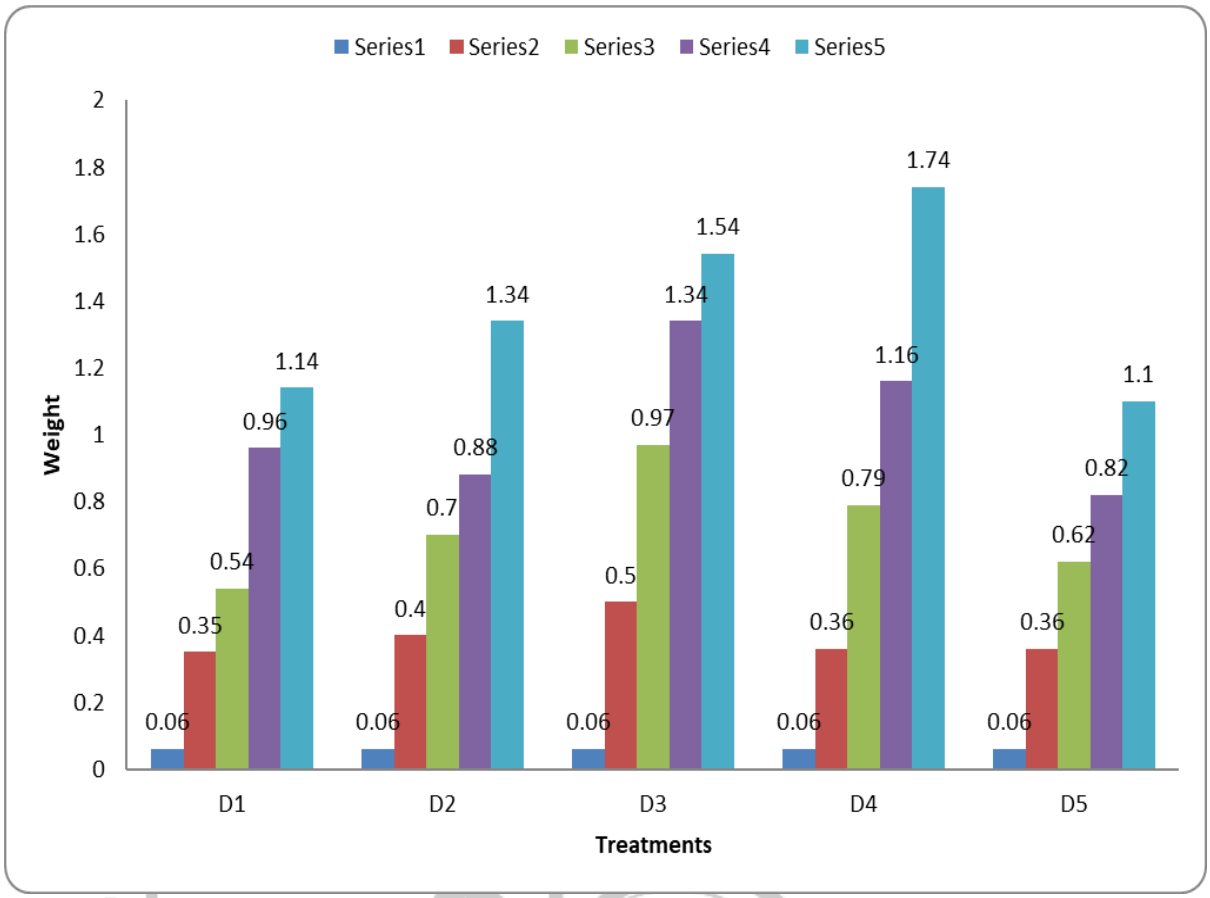
- **RESULTS AND DISCUSSION**
- The growth performance in length (cm) of *Oreochromis niloticus* treated with different volumes of honey based on the result obtained in 5 weeks, D_3 had the highest length with the value of 3.62 ± 1.42, followed by D_4, D_5 and D_2 respectively with the values of 3.46 ± 1.27, 3.09 ±1.05 and 2.78± 0.80 respectively. D_1 was observed to have the least growth performance in length with the value of 2.67 ± 0.65.However, higher length gain in the treatments with a similar percentage of honey used for the sex reversal of *Oreochromis niloticus* were same the present work though their experiment lasted for 10 weeks(Heriyati and Arfah 2012, Heriyati *et al.*, 2015).

Table 1: Growth performance in length (cm) of *Oreochromis niloticus* treated with different volumes of honey.

Treatment	Length
D_1	2.67 ± 0.65^{bc}
D_2	2.78 ± 0.80^{bc}
D_3	3.62 ± 1.42^{a}
D_4	3.46 ± 1.27^{ab}
D_5	3.09 ± 1.05^{b}

- Means with different superscripts are significantly different ($p<0.05$)
- **Keys:** D_1 0% honey, D_2 20% of honey mixed with 2 litres of water,
- D_3 30% of honey mixed with 2 litres of water, D_4 40% of honey mixed with 2 litres of water

Figure 1 showed the growth performance in weight of *Oreochromis niloticus* treated with different volumes of honey. From the figure below, it shows that all the treatments have an equal weight value of 0.06 in the first week of the experiment. However, from the second week to final week (i.e. the 5th week) reading there were variations. At the fifth week D_4 (30% of honey mixed with 2 litres of water) was observed to have the highest weight with a value of 1.74, followed by D_4, D_1, D_2 with 1.16, 0.96 and 0.88 respectively while D_1 (0% honey) with a weight value of 0.82.This result agree with the work of Abdullah 2018, that had higher growth in masculinisation of fish.



-

- Figure 1: Growth performance in weight of *O. niloticus* treated with different volumes of honey.

From the study, the percentage of male and female *Oreochromis niloticus* treated with different volumes of honey. D_4 (40% of honey mixed with 2 litres of water) was observed to have the highest percentage of male *Oreochromis niloticus* treated with honey with a value of 90% but it was also observed to have the lowest female percentage with 10%.This is in agreement with work on *Betta splendens* through honey immersion with 97% males and 3% females(Pattiasina *et al.,*2021) for 12 hours. This was followed by D_3, D_2, and D_5, with a male percentage value of 80%, 67% and 50% respectively while D_1 was observed to have the least male percentage with a value of 33% but also observed to have the highest female percentage with a value of 67%. The immersion of larvae with honey solution can work effectively for the process of male sex differentiation by looking at the relationship between the concentration of the honey solution and the time of immersion(Wahyuningsih *et al.,*2018). However, Pattiasina *et al.,*2021 stated that this depends on the type of fish used, the type of honey, and the age of the larvae.

-

Table 2:Percentage of males and females of *Oreochromis niloticus* treated with different volumes of honey.

Parameters	Percentage of male	Percentage of female
D_1	33% of male	67% of female
D_2	67% of male	33% of female
D_3	80% of male	20% of female
D_4	90% of male	10% of female
D_5	50% of male	50% of female

CONCLUSION

D_4(40%) of honey mixed with 2 litres of water) was observed to have the highest percentage of male and growth performance in length and weight. This volume of honey is therefore recommended for the production of male *O. niloticus*.

REFERENCES

Abdel-Fattah, M El-Sayed. (2002). Effects of stocking density and feeding levels on growth and feed efficiency of Nile tilapia (*Oreochromis niloticus*) fry. *Journal of Aquaculture research,* volume 33: 621-626.

Abdullah, N.(2018). Effects of Honey-induced Sex Reversal on Sex Ratio and Growth of Nile Tilapia(*Oreochromis niloticus*). *Nigerian Journal of Fisheries and Aquaculture* 6(1):1-6

Adebayo, O. T. and Popoola, O.M. (2008). comparative evaluation of efficacy and cost of synthetic and non synthetic hormones for artificial breeding of African catfish (*clarias gariepinus*, Burchell 1822). *Journal of Fisheries and Aquatic Science*, 3,66-71.

Carson F.L, Hladik C.2009. Histotechnology. A self-Instrustional Text. Hong Kong; ASCP *PRESS,3rd Edition,* 400p.

Chakraborty, S.B., Mazumdar, D., Chatterji, U. and Banerjee, S. 2011. Growth of mixed-sex and monosex Nile tilapia in different culture systems. *Turkish Journal of Fisheries and Aquatic Sciences*, 11(1): 131-138.

Chaves-Pozo, E.; García-Ayala, A. and Cabas, I. (2018). Effects of Sex Steroids on Fish Leukocytes. *MDPI Biology* 7(1): 9.

Contreras-Sanchez, W.M.; Fitzpatrick, M.S. and Schreck, C.B. **(**2001). Fate of Methyltestosterone in the Pond Environment: Impact of MT-contaminated soil on Tilapia sex differentiation. 9th Work Plan,Effluents and Pollution Research 2C (9ER2C), Final Report. In: Gupta A, McElwee K, Burright J, Cummings X and Egna H (Eds). 18th Annual Tec

Damayanti, A.; Sutresna, A. and Wildn, W. (2013). Aplikasi madu untuk pengarahan jenis kelamin pada ikan nila (*Oreochromis niloticus*). *Depik* 2(2): 82-86.

Fahmi AU 2018 Pengaruh Penggunaan Madu Terhadap Maskulinisasi Larva Ikan Mas (*Cyprinus carpio*) Secara Fenotip. Skripsi (Ambon: Program Studi Budidaya Perairan, Fakultas Perikanan dan Ilmu Kelautan. Universitas Pattimura)

Firmansyah, R.; Carman, O. and Soelistyowati, D.T. **(**2016). Ikan Pelangi Iriatherina werneri (Meiken, 1974) dengan Hormon Estradiol-17β. Jurnal Ikhtiologi Indonesia 16(3): 269-278.

Guerrero, R.D. and W. L. Shelton (1974): An Aceto-Carmine Squash Method for Sexing Juvenile Fishes, The Progressive Fish-Culturist, 36:1, 56-56

Hariyati,E, Arfat,A.H. and A.O. Sudrajat(2015). Aromatase gene expression of sex reversal of Nile tilapia(*Oreochromis niloticus* Linnaeus, 1758) using honey. *Jurnal Iktiologi Indonesia* 15(1):39-50

Heriyati, E. and Arfah,A. H. (2012).Sex reversal of Nile tilapia using honey and analysis of aromatase gene expression. Library of IPB University, Bogor, Indonesia Scientific Repository, MT – Fisheries [2289]

Kautsari, N.; Rahma, S. and Syafikri, D. (2015). Pengaruh Perendaman Larva dengan Berbagai Dosis Madu Sumbawa Terhadap Nisbah Jenis Kelamin dan Pertumbuhan Ikan Nila. *Jurnal Iktiologi Indonesia* 12(2): 99-106.

Lubis MA, Muslim and M Fitrani 2017 maskulinisasi ikan cupang (*Betta* sp.) menggunakan madu alam i melalui metode perendaman dengan konsentrasi berbeda. *Jurnal Akuakultur Rawa Indonesia*, **5**(1):97-108.

Mair, G.C., Abucay, J.S., Beardmore, J.A., Skibinski, D.O.F., (1995). Growth performance trials of genetically male tilapia (G17a-MT) derived from YY-males in *Oreochromis niloticus* L.: On station compositions with mixed sex and sex reversed male populations. *Aquaculture* 137, 313–322.

Megbowon, I. and Mojekwu, T.(2013). Tilapia sex reversal using methyltestosterone (MT) and its effect on fish, man and environment. *Biotechnology*, 13: 213-216.

Muslim M 2010 *Maskulinisasi Ikan Nila Oreochromis niloticus Dengan Pemberian Tepung Testis Sapi*. Tesis (Bogor: Institut Pertanian Bogor)

Nazar D U P, Basuki F, and Yuniarti T 2017 pengaruh lama waktu perendaman embrio dalam propolis terhadap maskulinisasi ikan cupang (*Betta splendens*). *Journal of Aquaculture Management and Technology*, 6(4):58-66.

Rosita L, Susilowati T and Nugroho R A 2018 Pengaruh lama waktu perendaman embrio dalam ekstrak purwoceng (*Pimpinella alpina*) terhadap pengalihan kelamin ikan cupang (*Betta splendens*). *Journal of Aquaculture Management and Technology*, 7(1): 120-127.

Pattiasina. B J, M M Pattinasarany, M M D Manuputty and E R Kokmesa(2021).Masculinization of beta fish larvae *Betta splendens* through the different treatment immersion of honey solution and larval age. *IOP Conf. Ser.: Earth Environ. Sci.* 797 012016

Pechsiri, J. and A. Yakupitiyage. 2005. A comparative study of growth and feed utilization efficiency of sex-reversed diploid and triploid Nile tilapia, Oreochromis niloticus L. Aquaculture Research, 36; 45-51

Soelistyowati, D.T.; Sudrajat, A.O. and Arfah, H. (2010). Maskulinisasi pada Ikan Nila Merah (*Oreochromis* sp.) Menggunakan Bahan Alami Resin Lebah Melalui Pakan Buatan. *Jurnal Akuakultur Indonesia* 9(2): 178–183

Sudrajat, A.O. and Sarida, M. (2006). Effectivity of aromatase inhibitor and 17a-metiltestosteron treatments in male production of freshwater prawn (*Macrobrachium roserbergii* de Man). Jakarta, Aquaculture Indonesian.

Wahyuningsih H, Rachimi and Prasetio E (2018). Efektivitas madu lebah terhadap jantanisasi (maskulinisasi) dengan metode perendaman pada larva ikan nila merah (*Oreochromis* sp.). *Jurnal Ruaya* 6(1): 23-9