EFFECT OF ELEVATED TEMPERATURE TREATMENT AND PAWPAW SEED POWDER ON PRODUCTION OF STERILE MONOSEX NILE TILAPIA

BY

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DECLARATION

DECLARATION BY THE CANDIDATE

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I dedicate this thesis to my husband Mr. John Wachira and my daughter Hellen Nyambura.

ABSTRACT

Nile tilapia is the most widely cultured fish species in Kenya. The greatest problem with tilapia is its prolific breeding due to mixed sex, resulting in stunted growth of the fish. Therefore, there was a need to employ simple techniques that embrace use of locally available materials for ease of implementation, which previous methods did not fully consider. This study therefore investigated the effect of high temperature treatment and pawpaw seeds powder on production of sterile monosex Nile tilapia (*Oreochromis niloticus*). The effect of high temperature treatment on sex differentiation and survival rate at different larval stages was studied; as well as the effect of pawpaw seeds powder on the histological structure of gonads, growth performance and proximate composition of *O. niloticus*. The research was carried out in two phases with 12 glass aquaria in each phase. In Phase 1 temperature treatment at 36 ^{o}C was done for 10 days at different days post-fertilization (dpf) of 1 dpf, 6 dpf and 10 dpf. The control was kept at 28 ^{o}C from the egg stage to the end of the experiment. In Phase 2 administration of pawpaw seeds powder to the fish from the best result of Phase 1 in terms of highest male ratio which was 10 dpf was done at 0g, 4g, 8g and 12g PSP/kg feed for a period of 60 days. Male percentage ranged from 45.56-82.22% while female percentage ranged between 17.78-54.44%. The control group had the least percentage of males (45.56%) while swim-up fry had the highest percentage of males (82.22%). Percent Survival was lowest at egg stage (43%) and highest at control group (80.7%). In Phase 2, histology of gonads of fish treated with different levels of PSP revealed that ovaries and testes of 0g PSP/kg feed were normal. Ovaries subjected to 4g PSP/kg feed had degenerative stromas while testes had scanty spermatozoa. At 8g PSP/kg feed, the ovaries showed increased atretic follicles and testes had degeneration of spermatozoa. Treatment with 12g PSP/kg feed resulted in severe atretic follicles of the ovaries and deformation of seminiferous tubules and erosion of spermatozoa of the testes. Proximate composition of the fish carcass showed that the 8g PSP/kg feed had the highest values (mean ± SE) of CP and ash (53.97 ± 0.094 and 20.05 ± 0.35) respectively. Body weight gain and Specific growth rate was highest at the 8g PSP/kg treatment level, but this treatment level had the lowest Feed Conversion Ratio. This study recommended the use of high temperature treatment of 36 ^{o}C to yield a high male percentage and this technique can be combined with the administration of pawpaw seeds powder to control the breeding of *O. niloticus* in production units.

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LIST OF ABBREVIATIONS

AOAC :	Association of Official Analytical Chemists
BWG:	Body weight gain
CP:	Crude protein
DM:	Dry Matter
DO:	Dissolved Oxygen
Dpf:	Days post-fertilization
ESD:	Environmental sex determination
FAO:	Food and Agriculture Organization
FCR:	Feed Conversion Ratio
GSD:	Genetic sex determination
H&E:	Hematoxylin and Eosin
PSP:	Pawpaw seeds powder
SGR:	Specific growth rate
TE:	Temperature effects
TN:	Total Nitrogen
TSD:	Temperature sex determination
USA:	United States of America
V_E:	Environmental Variance
V_G:	Genetic Variance

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CHAPTER ONE

INTRODUCTION

Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758) is a well-studied, fast growing and widely cultured species. It has a wide range of distribution in both the tropics and sub-tropical regions. It is currently ranked second in global production after carps and is likely to be the most important cultured fish in the 21st century (Ridha, 2006). It is the most dominant fish among the tilapias that are farmed in sub-Saharan Africa, either for subsistence or export (Pradeep *et al*; 2012). Tilapia display many favourable attributes as culture species, on the basis of its general hardness, resistance to diseases, high yield potential and ability to grow on a wide range of natural and cheap artificial foods, as well as good consumer acceptance (FAO, 2012). Additionally, it can also withstand low oxygen concentrations, overcrowding, tolerate difficult ecological conditions and a wide range of salinities and still produce a highly acceptable flesh (El-Sayed, 2006). Tilapia can grow up to 500 – 600g in 6 - 8 months and may reach sexual maturity as early as 2 – 3 months of age in culture. They spawn naturally throughout the year and very little management effort is required to breed these fish (Lim and Webster, 2006). Cultured *O. niloticus* are easily bred in captivity without complex hatchery equipment or hormonal induction to make them spawn and they breed throughout the year in the tropics. Tilapia farming therefore, if well developed, could provide the needed fish protein as well as monetary gains to fish farmers (Angienda *et al*; 2010).

Some fish species do not reproduce in the culture environment before reaching market size but Tilapias are a paradox in reproduction since they attain early maturity and

undergo prolific breeding. This often leads to overpopulation of production ponds with stunted fish (Jegede and Fagbenro, 2008). Overpopulation results from the uncontrolled reproduction, which in turn leads to stunted growth, with normal production of fish with low nutritional and commercial values. Several methods have been used to control such undesirable tilapia population including monosex culture (Angienda *et al*; 2010), sex reversal preferably to get all males (Megbowon and Mojekwu, 2014), sterilization (Nakamura *et al*; 2015), cage/tank culture (FAO, 1978), use of predators (Fagbenro, 2004), high stocking density (Mensah *et al*; 2013), intermittent/selective harvesting and use of slow maturing tilapia species (Mair and Little, 1991). Commercial production of tilapia often relies on monosex culture of males. Sexuality of fish has great significance in aquaculture due to the differences in growth rates, survival rate, size, behavior pattern and breeding time (Manosroi *et al*; 2004). In *O. niloticus,* the culture of all male population is important for higher growth rate and more uniformly sized fish (Sotozarazua *et al*; 2011). This is because *O. niloticus* displays dimorphic sexual growth in which males grow and reach a larger ultimate size faster than the females (Guerrero and Guerrero, 2001). Taking these aspects into account, considerable work has been done regarding the techniques used for reversing sex in *O. niloticus*, its growth performance and meat quality (Manteen, 2007). The production of a single sex population, especially of male offers several advantages in *O. niloticus* culture including enhanced growth, uniform size at harvesting and prevention of precocious reproduction. Four principal methods of producing monosex Tilapia include: Manual sexing, hybridization including environmental manipulation, genetic manipulation and hormonal sex reversal (Fuentes-silva *et al*; 2013).

Although cases of temperature influence on sex ratio are numerous and widely reported, many other environmental factors have been implicated in influencing sexual phenotype and sex ratio. These include pH (Rubin, 1985), stocking density (Huertas and Cerda, 2006), pollution (Fryxell *et al*; 2015) and competitive suppression of reproductive function (probably through feedback mechanisms on neuroendocrine pathways) and other social conditions (Baroiller *et al*; 2009).

Sterile populations can reduce unwanted reproduction or improve growth rate by energy diversion from gametogenesis, and associated behaviours. The use of biological inhibitory agents to induce sterility has been advocated (Ekanem and Okoronkwo, 2003). Plants with antifertility properties have been thought of as solution since they can easily be obtained and incorporated in Tilapia feed. Some Plants that have been tested and proven for antifertility properties in tilapia include: Pawpaw seeds (Ekanem and Okoronkwo, 2003) Neem (Jegede and Fagbenro, 2008) and Aloe vera (Kushwaha, 2013). Other plants which have been tested for antifertility in other animals such as rats include Mango leaves (Zhang *et al*; 2014), guava leaves (Ekaluo *et al*; 2013), Jatropha (Jain *et al*; 2013) and lesser yam (Shajeela *et al*; 2011). These plants have been shown to cause no harm to the test fish, environment and humans, and are therefore universally acceptable for use in inducing sterility (Gupta and Sharma, 2006).

Carica papaya is a common human fruit; available throughout the year in the tropics. It contains medicinal properties and the major active ingredients recorded include, carpine, chymopapain and papain, a bactericidal aglycone of glucotropaeolin, benzyl isothiocyanate, a glycoside sinigrin, the enzyme myrosin, and carpasemine (Jackwheeler,

2003). Seeds of papaya account for about 16% of the fresh fruit weight (Passera and Spettoli, 1981). According to Bolu *et al.* (2009), proximate analysis of dried pawpaw seeds contains 97.27% dry matter of which 30.08% is crude protein, 34.80% crude fat, 1.67% crude fiber, 7.11% ash and 23.67% nitrogen free extract. The seeds also contain numerous carbohydrates, fatty acids, an enzyme carpasemine, and a plant growth inhibitor caricacin (Casey, 1960). The fat content, on a dry weight basis, was 60% in papaya endosperm (Passera and Spettoli, 1981). Chinoy *et al.* (1997) reported that oleic, palmitic, stearic and linoleic acids are present in the seeds. Therefore, many researchers used pawpaw seeds powder (PSP) as a natural reproductive inhibitor in *O. niloticus* (Ekanem and Bassey, 2003; Ekanem and Okoronkwo, 2003; Jegede and Fagbenro, 2008 and Abbas and Abbas, 2011). On the other hand, some of results showed that adding of PSP in diets caused delay of growth and high percentage of mortality in *O. niloticus* fry (Ekanem and Okoronkwo, 2003; Ayotunde and Ofem, 2008 and Abbas and Abbas, 2011).

The study therefore set out to determine effects of elevated temperature on *O. niloticus* masculinization at various days post fertilization and also to find out the effects of PSP in controlling the prolific breeding of the species.

1.1 Problem Statement

Oreochromis niloticus do not reach their full aquaculture potential due to their early maturity and prolific breeding (Paradeep *et al*; 2011). This problem often leads to overpopulation in production ponds leading to stunted fish growth and low market value (Jegede and Fagbenro, 2008). Several methods have been used to control such

undesirable tilapia population including monosex culture, sex reversal (preferably to get all males), sterilization, cage/tank culture, use of predators, high density stocking, intermittent/selective harvesting and use of slow maturing tilapia species. These methods that are currently used have major technical drawbacks that make it difficult for farmers to adopt and develop. Sterilization using chemosterilants and irradiation, for example, has disadvantages of expensive technology, hatchery facilities and requires skilled labour; sex reversal by use of hormones is expensive, the hormone is difficult to obtain due to regulations and consumer resistance to hormone-treated fish when compared to that of local plant extracts (Jegede and Fagbenro, 2008). Furthermore, synthetic hormones accumulate in the sediment, water and aquatic biota and can harm non-target organisms (Cek *et al*; 2004).

A variety of other methods have also been used to produce all male fingerlings, including interspecific hybridization and YY supermales (Ezaz *et al*; 2005; Baroiller et al; 2008). However, all these methods have drawbacks and very few have progressed beyond experimental studies or development trials to widespread adoption by farmers. These methods are not universally applicable, in part because of their technical complexity. Production of the YY supermales is one feasible technique that produces superior males with desirable characteristics, but it is laborious, requires expertise and prolonged period to attain the supermales (Phelps and Popma, 2000; Ezaz *et al*; 2005; Baroiller *et al*; 2008). Additionally, monosex culture by manual sexing is laborious and requires expertise and accuracy. According to Fagbenro (2000), the use of local predatory fish species to control undesirable Tilapia recruitment in ponds is one of the most effective and practical methods where a thorough assessment of users (farmer and consumer)

perspectives are considered. Even with the use of predators, the main drawback remains the excessive recruitment in ponds, which result in low yields of harvestable size (Mair, 2001).

Even with a variety of methods in place for monosex culture of *O. niloticus*, the problem of mixed sex in the culture systems still occur to some extent, because no single method attains all single sex population, whether males or females. Therefore, this implies that the problem of massive reproduction and stunted growth still occur. There is thus a need to employ a combination of techniques that will yield a sterile population. These methods should be technically easy and embrace use of locally available materials for ease of implementation, which previous methods did not fully consider.

1.2 Justification

Research to curb the problems of prolific reproduction and stunted growth in *O. niloticus* should be geared towards giving achievable solutions which farmers can implement for sex reversal and sterilization. Hence, there is need to use easier techniques to obtain all or almost all male population. Though different methods of treatment of fish for sex reversal have been experimented, the incorporation of hormone through oral administration has been successful to a greater extent than any other method. But invariably, there has been perceived environmental and health issues related to hormone use, that is, possible effects of treatment residues on water quality, biodiversity and consumers' health. Also, considering the growing concerns for food security, finding an effective sex control alternative in fish remains an urgent challenge for aquaculture. This must be non-hazardous to consumers and environmentally friendly method. In the USA, sex reversal of tilapia is a licensed procedure, while in the European Community the direct use of

hormones is banned (Cek *et al*; 2004). More environmentally friendly methods and materials should therefore be developed in order to continue the use of direct sex reversal.

The quest for easy monosex production techniques and discovering natural plants that will keep the viability of the sex reversal method as well as eliminate the skepticisms on synthetic hormonal treatments becomes a highly placed option in increasing tilapia productivity. Heat treatment technology, where the fry are subjected to heat shock to trigger development of males, is an easy technique that farmers can easily adopt. In order to prevent prolific reproduction in case sex reversal does not yield all males, sterility should be induced. Sterile populations can reduce unwanted reproduction or improve growth rate by energy diversion from gametogenesis, and associated behaviours. The use of biological inhibitory agents to induce sterility is being advocated (Ekanem and Okoronkwo, 2003). Plants with antifertility properties have been thought of as a possible solution since they are easily obtained and incorporated in Tilapia feed.

This study investigated the potential of elevated temperature treatment technique to yield a monosex population of *O. niloticus* followed by the use of locally available plant extract of pawpaw seed to induce sterility so as to control their prolific reproduction breeding.

1.3 Objectives

1.3.1 General objective

To produce monosex sterile *Oreochromis niloticus* using elevated temperature and papaya seeds (*Carica papaya*) extract.

1.3.2 Specific objectives

i. To determine the effect of elevated temperature treatment on sex ratios of *O. niloticus* at three different larval stages.

ii. To determine the effect of elevated temperature treatment on the percent survival of *O. niloticus.*

iii. To determine the effect of pawpaw seed powder on the histological structure of gonads of *O. niloticus*.

iv. To determine the effect of Pawpaw seed powder on the growth and proximate composition of *O. niloticus* carcass.

1.4 Hypotheses

i. Exposure of larval stages of *O. niloticus* to elevated temperatures has no effect on sex ratios.

ii. Survival rate of larval stages of *O. niloticus* is not affected by elevated temperature.

iii. Feeding of *O. niloticus* with pawpaw seed powder has no effect on the histological structure of the gonads.

iv. Feeding of O. niloticus with pawpaw seed powder has no effect on the growth and proximate composition of carcass.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sex determination in fish

According to Hayes (1998), sex determination is the mechanisms directing sex differentiation while sex differentiation is the development of testes or ovaries from the undifferentiated gonads. Sex determining mechanisms in gonochoristic fish can broadly be classified as genotypic (GSD), temperature-dependent (TSD), or genotypic plus temperature effects (GSD + TE) (Ospina and Piferrer, 2008; Bachtrog *et al*; 2014). Sex determination in fish is characterized by extraordinary variation, including genetic and/or environmental sex determination, male or female heterogamety, single gene and polygenic systems, protandry, protogyny and simultaneous hermaphroditism as well as social influence on sexual determination. This has been observed in *O. niloticus*, Common Carp (*Cyprinus carpio*), Rainbow trout (*Oncorhynchus mykiss*), Zebrafish (*Danio rerio*), Medaka (*Oryzias latipes*), Japanese flounder (*Paralichthys olivaceus*), Atlantic silverside (*Menidia menidia*) and European bass (*Dicentrarchus labrax)* (Baroiller *et al*; 2009). In each of these species, however, environmental influences and autosomal modifier genes can also dictate sex determination (Siegfried, 2010). Sex-determination events primarily function during early development to set the course of gonadal development. Environment factor alterations (temperature, hormone treatment, hypoxia, population density, and pH) are known to influence phenotypic sex in several fish species. They can either determine the sex or influence the sex differentiation. For fish species with TSD, they should have a sex ratio in response to temperature within the

range of temperature during development in the wild and should not have sex chromosomes (Ospina and Piferrer, 2008). However, artificially high or low temperatures during critical thermosensitive periods, which is ecologically irrelevant, also results in sex ratio changes in many fish, which are defined as the GSD + TE type. For GSD + TE, a high or low temperature can override the influence of genetics and switch the sex determination mechanisms when the gonads are undifferentiated (Tessema *et al*; 2006).

Oreochromis niloticus sex is determined by genetic factor (Genetic Sex Differentiation - GSD), by temperature (Temperature Sex Differentiation - TSD) or by interaction of temperature and genotype (Wang *et al*; 2014). Bezault *et al* (2007), confirmed that genotype-environment interactions play a key role in temperature induced sex determination in *O. niloticus.* The complex sex determining system in *O. niloticus* was determined by the interactions between a genetic and the temperature influence. Tessema *et al* (2006) also reported the linkage between temperature treatment and heritability of temperature sex inversion in tilapia with the Y gene being responsible for determination of sex inversion in temperature treatments. The effectiveness of temperature treatments in the masculinization of fish has also been traced to increased stress level leading to higher blood cortisol levels and associated masculinization although the mechanism is unclear (Martínez *et al*; 2014).

Previous studies have indicated that temperature is an important factor in sex determination of *O. niloticus* during the early stages of development but do not clearly give the specific critical development stage(s) when this determination occurs.

2.2 Temperature manipulation effects on sex ratio in fish

Temperature is the main environmental factor influencing the sex ratio in fish (that is, temperature dependent sex determination — TSD). This was first demonstrated in *M menidia* (Conover, 1984). Then, parental strain and/or population influences (genotype-temperature interactions) of temperature effects on sex ratio were confirmed in several fish species with sexual dimorphic growth patterns, for example, *L. macrochirus* (Shen *et al*; 2016), *O. niloticus* (Angienda *et al*; 2010), *O. mykiss* (Valdivia *et al*; 2014), *D. labrax* (Blaquez *et al*; 1998) and turbot (*Scophthalmus maximus*) (Haffray *et al*; 2009). Therefore, in fish, sex differentiation and sex inversion can be influenced by environmental factors with temperature being the most important factor. Some important farmed species have sex-related growth, for example, *O. niloticus*, sea *D. labrax* and the bastard halibut (*Paralichthys olivaceus*). It is important to reveal the sex determination process and control the sex ratio in these species considering economic aspects of aquaculture. Other environmental conditions such as hormonal treatments, fish density, pH and hypoxia have also been shown to influence the sex ratio of fish species from very divergent orders (Zhang *et al*; 2009).

The underlying mechanism of temperature sex determination (TSD) has been related to a suppression of aromatase (cyp19) expression at male promoting temperatures resulting in masculinization, and an increased expression of the aromatase in ovaries at feminizing temperatures (Baroiller and D'Cotta, 2001; Karube *et al*; 2007). Male gonad differentiation can be achieved by applying high temperature treatment during the critical period of sex differentiation (Aulia *et al*; 2015). The change in sex of tilapia from female

to male increases with increase in temperature while ovarian differentiation is induced by low temperatures (Baroiller and D'Cotta, 2001). Increased water temperature during sex differentiation favours testicular development hence a greater male population (Tessema *et al*; 2006). It is possible to masculinize XX progenies (100% females) with elevated temperatures above 32°C, giving functional male phenotypes. Gonad differentiation of progenies of some fish species can be directed toward testicular differentiation by high temperature treatment applied at 10 days post fertilization. High temperatures can efficiently masculinize some progenies if started around 10 days post fertilization and if applied for at least 10 days, with longer periods being just as effective. However, if a treatment is applied for a 10-day period but begins at 7 days post fertilization, it has no effect on sex ratios (Carlos *et al*; 2012). A considerable amount of previous research has made temperature sex determination mechanism in *O. niloticus* relatively clear (Li *et al*; 2015). In *O. niloticus*, a GSD + TE sex determination fish, high temperature treatment applied after hatching (around 10 days post-fertilization) and lasting from 10 to 28 days, significantly increased the male ratio (Dang *et al*; 2011). Therefore, the thermal control of sex in *O. niloticus* is an economic and environmentally friendly method (Martinez *et al*; 2014). The ability of a fish species to respond to temperature induced sex reversal must be heritable and traceable to parental stock hence it is an evolutionary trait that involves a V_E (Environmental variance - Temperature) and genotype interaction (V_G) which is revealed in family sex ratios as a result of strong parental effects (Shen and Wang, 2014).

Rougeot *et al* (2008) reported the pre-hatching induction of masculinization during embryonic development via temperature treatment of fertilized eggs with a relatively low

success rate compared to dietary administration of hormones as well as temperature treatment post hatching. Tessema *et al*; (2006) reported that masculinization rate did not increase with increasing temperature from 36^{o}C to 38^{o}C but strain response to elevated temperature treatment differed while Rougeot *et al* (2008) confirmed that elevated temperature treatment affects survival more than temperature duration.

Studies in most fish species used domestic strains reared under controlled conditions. In *O. niloticus* and sea bass, domestic stocks and field-collected populations showed similar patterns of thermosensitivity under controlled conditions. In tilapia, transitional forms within a genetic sex determination (GSD) and environmental sex determination (ESD) continuum seem to exist. Temperature regulates the expression of the ovarian-aromatase cyp19a1 which is consistently inhibited in temperature masculinized gonads. Temperature through apoptosis or germ cell proliferation could be a critical threshold for male or female sex differentiation (Baroiller *et al*; 2009). In most thermosensitive species (some Atherinids, Poecilids, Cichlids: tilapias, goldfish, a Siluriform, a flatfish ellipsis) male to female ratio increases with temperature and/or ovarian differentiation is induced by low temperatures. Conversely, in some rare species (*Dicentrarchus labrax, Ictalurus punctatus*), high temperatures may produce female-biased sex ratios and/or low temperatures promote male-biased sex ratios. In the hirame *Paralichthys olivaceus*, both high and low temperatures induce monosex male populations while intermediate temperatures yield a 1:1 sex ratio. Fish show discriminations in their TSD patterns since monosex populations are generally not produced at extreme temperatures, suggesting the existence of strong temperature-genotype interactions. In fish displaying TSD,

temperature treatments must be applied at a critical sensitive period, relatively similar to the hormone sensitive period (Barroiler *et al*; 1996).

Studies have reported evidence of sex manipulation in *O. niloticus* at high temperatures towards males. Angienda *et al* (2010) for example, reported 86.31% males after heat treatment at 36 ^{o}C for ten days. However, studies focusing on the effect of high temperature on sex ratios at different larval stages are lacking.

2.3 Effect of temperature on survival of fish

Eggs have a much lower thermal tolerance. External temperatures govern the biological processes of eggs and the embryos inside. As the water gets warmer their metabolism increases, demanding more and more oxygen. Unlike juvenile and adult fish, eggs cannot move and do not have a developed respiratory or circulatory system. Instead they rely on flowing water to supply oxygen and carry away waste products. *Oreochromis niloticus* is thermal tolerant; these fish do not grow well at temperature below 16°C and cannot usually survive for more than a few days below 10°C, but they are remarkably tolerant of high temperatures, up to 40-42°C (Chervinski, 1982). Dan and Little (2000) assessed the performance of *O. niloticus* broodstock of the Thai, GIFT, Egypt and Vietnam strains over- wintered in deep and shallow ponds, as well as in deep and shallow hapas suspended in a single deep pond for evaluation of the influence of over-wintering systems on the survival and growth rates of fish. Large (>1g) and small (<1g) tilapia seed were over-wintered in deep hapas in ponds for comparison of their survival performance. The survival rate of larger monosex tilapia fry was 54%, which was significantly ($p < 0.05$) higher than that of smaller fry.

A study by Angienda *et al* (2010) reported that the maximum temperatures that provide for survival of fry lies within a narrow range of 36 ± 0.5°C, resulting in 65.25% survival of the fry. Higher temperatures of 37 °C resulted in low survival of fry.

2.4 Application of pawpaw seeds extract as a reproductive inhibitor in fish

In the recent years, medicinal plants have successfully been used to induce sterility in laboratory animals. One of the plants is pawpaw (*Carica papaya*) whose seeds have been used as fertility control agents in some laboratory animals such as rats (Udoh *et al*; 2008) and langur monkeys (Lohiya *et al*; 2002). In the same trend, many researchers have used pawpaw seed powder (PSP) as a natural reproductive inhibitor in *O. niloticus*. Most of the studies used pawpaw seeds as reproductive inhibitors in adult *O. niloticus* after sexual maturation (Ekanem and Okoronkwo, 2003). The obtained results of these studies showed that the larval stages of many teleost species contain both ovarian and testicular tissues, with sexual differentiation commencing shortly after hatching or after the initiation of feeding. Generally, the obtained results show that the high levels of PSP (6 and 8 g PSP /kg diet) on long exposure periods (45 to 60 day) in diets of Nile tilapia after hatching gave the positive effect to control of the reproductive process in *O. niloticus* through decreased sex hormone (testosterone and progesterone) and caused several histological alternations in testes and ovaries, which reduced fertility in both males and females *O. niloticus* (Sotozarazua *et al*; 2011). Jackwheeler (2003) reported that Pawpaw seeds contain active ingredients such as caricacin, an enzyme carpasemine, a plant growth inhibitor, and oleanolic glycoside. The study further reported that papaya contains antifertility properties, particularly the seeds. A complete loss of fertility has been reported in male rabbits, rats and monkeys fed an extract of papaya seeds. Lohiya *et al*

(2002) further suggested that ingestion of papaya seeds may adversely affect the fertility of human males or other male mammals. Ekanem and Okoronkwo (2003) reported high success in using pawpaw seed powder in inducing sterility in male *O. niloticus* when administered through feed. However, the toxicity and effectiveness of *C. papaya* to aquatic organisms, particularly fishes, have not been examined.

In Nigeria, Ekanem and Okoronkwo (2003) used *C. papaya* seed as fertility control agent on male *O. niloticus*. Mature male tilapia with mean weight 40 g were treated for 30 days with 4.9 g/kg/day (low dose) and a 9.8 g/kg/day (high dose) of ground pawpaw seeds incorporated into their feed. In order to determine the effect of the treatments, a control treatment was added using fish of similar sizes fed with feed that did not contain pawpaw seed. No spawning occurred in any of the replicates in the high dose treatments during the 30-day treatment period. Fish in the control experiment spawned two weeks and five weeks after while fish in the low dose treatment spawned three weeks after the treatment was discontinued. When sections of the testes were examined histologically, swollen nuclei in the low dose treatment and disintegrated cells in the high dose treatment were observed. This indicated the positive effect of pawpaw seeds in inducing sterility in *O. niloticus*. The application of this method of controlling reproduction in tilapia is straightforward and can easily be adopted by fish farmers in as much as pawpaw seeds are available all year round in the tropics and subtropical regions and even in Kenya.

2.5 Effect of pawpaw seeds powder on the growth performance and proximate composition of fish

Studies have reported that the dietary PSP at level 6 g/ kg diet for 45 day after absorbing the yolk sac of *O. niloticus* fry may be used as a growth promoter for *O. niloticus*, which improved most of the growth performance parameters, survival, food conversion ratio (FCR) and fish body composition (Sotozarazua *et al*; 2011). On the other hand, some of the results showed that adding of PSP in diets caused delay of growth and high percentage of mortality in *O. niloticus* fry (Sotozarazua *et al*; 2011). PSP was observed to promote growth performance, improve food conversion ratio, survival rate, boost stress resistance (Jegede and Fagbenro 2008; Abdelhak *et al*; 2013) and act as immunostimulants (Pandey *et al*; 2012) when the feed was administered to the fry two weeks after hatching (Farrag *et al*; 2013). The concept of using pawpaw as a reproductive control agent and growth enhancer in fish farming sounds sustainable and can be adopted by fish farmers since the papaw fruits are available throughout the year in tropical and subtropical regions. Pawpaw seeds are believed to possess phytochemicals that enhance the digestive systems, feed utilization capacity and ultimately improve the growth performance of the fish (Iipinge *et al*; 2019).

According to Baroiller *et al* (2009), proximate analysis of dried pawpaw seeds contains 97.27% dry matter, 30.08% crude protein, 34.80% crude fat, 1.67% crude fiber, 7.11% ash and 23.67% nitrogen free extract. The seeds contain proteins, carbohydrates, fatty acids, an enzyme carpasemine, and a plant growth inhibitor caricacin. The fat content, on

a dry weight basis, was 60% in papaya endosperm. Oleic, palmitic, stearic and linoleic acids are reported to be present in the seeds.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out at the University of Eldoret fish hatchery in the Department of Fisheries and Aquatic Sciences for a period of 8 months from November 2016 to June 2017. The university is located in Uasin Gishu County, 9 Km north east of Eldoret Municipality, on the Eldoret - Ziwa road in Rift valley, Kenya. It lies 0°35°N and 35°N-12°E at an altitude of 2180M above sea level. Temperatures range from 8.4 °C - 25 °C, with bimodial rainy seasons ranging between 900mm to 1,200mm per annum.

3.2 Source of experimental fish

The experimental fish were obtained from the University of Eldoret fish farm. Sexually mature *O. niloticus* brooders were selected and transported to the hatchery and acclimatized in in plastic holding tanks for two weeks in the hatchery where the study was conducted. Acclimation to the tank environment lasted for two weeks during which the fish were fed with a formulated feed containing 35% crude protein purchased from Jambo fish farm in Mumias, Kenya. After two weeks the brooders were mixed at the ratio of three females to one male and let to brood freely, after which fertilized eggs were collected from the mouth of the female and incubated in the aquaria.

3.3 Study design

The research employed a completely randomized design (CRD) with three replicates per treatment. The experiment was conducted indoors in two phases with 12 glass aquaria in each phase. Fertilized *O. niloticus* eggs were collected from the mouth of the female

brooders using plastic buckets and divided into four batches, designated as B1, B2, B3 and B4 with three replicates for each batch and incubated separately in twelve hatching aquaria. Each hatching aquaria had two hundred eggs. They were observed daily for hatching and yolk sac absorption. The number of fry in each aquarium was reduced to thirty after hatching so as to have an equal number of fry in all quaria. One day after yolk adsorption constituted day one post yolk sac stage of development. Light feeding on mashed feeds was also commenced at this time.

Phase 1: Elevated temperature treatment– This was done at 36^{o}C for 10 days at egg stage (B1 - 1 dpf); one day post-hatch (yolk sac fry -B2: 6 dpf); and one day after the absorption of the yolk sac (swim up fry -B3: 10 dpf). B4 was the control and was kept at 28 ^{o}C from the egg stage to the end of the experiment. Submersible thermal controlled electric heaters set at 36^{o}C were used for the treatment with elevated temperature. After finishing the elevated temperature treatments all groups were kept at the control temperature (28 ^{o}C) for six months. Continuous aeration was supplied in all treatments throughout the experiment. Fish were fed a commercial diet of 35% Crude Protein throughout the phase 1.

B1 (egg stage): 1 dpf	B2 (yolk sac fry): 6 dpf	B3 (swim up fry): 10 dpf	B4 (control)	B1 (egg stage): 1 dpf	B2 (yolk sac fry): 6 dpf
B3 (swim up fry): 10 dpf	B4 (control)	B1 (egg stage): 1 dpf	B2 (yolk sac fry): 6 dpf	B3 (swim up fry): 10 dpf	B4 (control)

Figure 3.1: Stages of exposure in terms of days post-fertilization to elevated temperature of 36 ^{o}C during Phase 1 of the study.

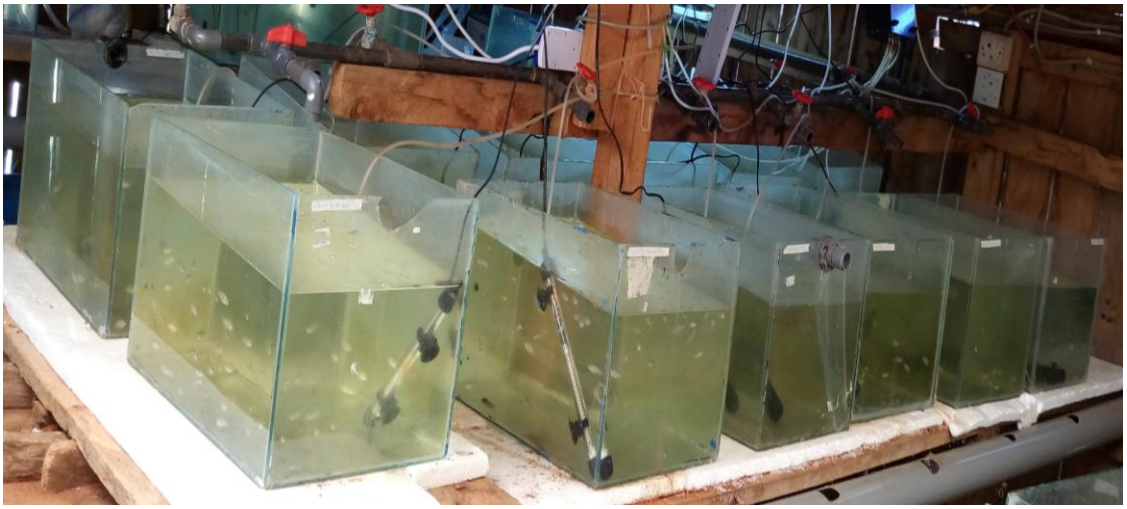


Figure 3.2: Photo showing the experimental set-up of Phase 1 of the study.

(Source: Author, 2017)

Phase 2: Administration of pawpaw seeds powder to the fish - The best result from temperature treatment (that is; the treatment that resulted in the highest percentage of male – which was swim -up fry that had been treated at 10 dpf) was subjected to treatment with pawpaw seeds powder from one day post-absorption of yolk sac. Phase 2 was not a continuation of phase 1, but rather a repeat of treatment with elevated temperature of swim-up fry at 10 dpf, then feeding the fry with PSP. A commercial diet of 35% Crude Protein was locally purchased from Jambo fish farm in Mumias, Kenya and mixed with different quantities of pawpaw seeds. Twelve aquaria were used for treatment with pawpaw seeds. The treatments were done at four different levels (0g, 4g, 8g and 12g/kg feed) of pawpaw seeds with three replicates for each treatment and begun one day after the absorption of the yolk sac for a period of 60 days. Each aquarium had thirty fry. Fry were fed at 4% body weight/day in four instalments daily at 0900h, 1100h, 1300h and 1500h, for 60 days.

0g PSP/kg feed	4g PSP/kg feed	8g PSP/kg feed	12g PSP /kg feed	0g PSP/kg feed	4g PSP/kg feed
8g PSP/kg feed	12g PSP /kg feed	0g PSP/kg feed	4g PSP/kg feed	8g PSP/kg feed	12g PSP /kg feed

Figure 3.3: Inclusion levels of PSP in the experimental diets fed to the fish during Phase 2 of the experiment.

Figure 3.4: Fresh fruits and dried seeds of *C. papaya* used for the preparation of experimental feed of PSP used in Phase 2 of the experiment. (Source: Author, 2017)

3.4 Sampling of fish

At the end of phase 1, 2 fish from each treatment were sampled for histological examination of gonads to identify their sex. During Phase 2, a sample of 20 fish from each aquarium was taken fortnightly and their length and weight measured. Fish were removed from each tank using a minnow seine, and returned to the tank following measurement. Electronic balance (readability 0.01 g) was used to record fish weight and

a meter ruler to the nearest 0.1 cm was used to measure the length. 5 fish were taken from each aquarium at the end of Phase 2 for histological examination of the gonads to examine gonadal structure.

3.5 Water quality monitoring

Water quality parameters were monitored throughout the study period. Parameters that were measured include Dissolved oxygen (DO), temperature and pH. Water temperature, DO and pH were measured using a thermometer, an oxymeter (YSI 200) and a portable field pH meter respectively daily. DO was maintained at 4-6 mg/l; pH 6.5-7.5 and temperature was maintained at 28±0.5°C in the control experiment, and in the treatments at 36°C for 10 days during the high temperature treatments.

3.6 Proximate analysis of fish carcass

At the end of the experiment, the carcass of the control and experimental *O. niloticus* subjected to pawpaw seeds powder were assayed for proximate composition using the AOAC (1990) methods for the composition analysis of crude lipids, Dry Matter (DM), crude protein (CP) and ash contents. Ten fish from each aquarium were collected and oven-dried for proximate composition determination as follows: DM, by determining the weight differences before and after oven drying at 105 ^{0}C until a constant weight was obtained; crude lipids, by Soxhlet extraction with ether; ash, by incineration in a muffle furnace at 550 ^{0}C for 8 hours; and CP, by the Kjeldahl method after acid digestion to obtain Total Nitrogen (TN), a factor of 6.25 was used for converting TN to CP of the fish. The following formulas were used to calculate the above contents:

$$Dry\ Matter = \frac{Weight\ after\ drying\ (g)}{Weight\ before\ drying\ (g)} \times 100$$

$$Crude\ Lipid = \frac{Weight\ of\ sample\ (g) - Fat\ free\ residue\ (g)}{Dry\ Matter\ (g)} \times 100$$

$$Crude\ Protein = Total\ Nitrogen\ Concentration \times 6.25$$

$$Ash\ (\%) = \frac{Ash\ (g)}{Dry\ Matter\ (g)} \times 100$$

3.7 Measurement of fish growth and percent survival

Dead and unfertilized eggs were removed every 24 hours to avoid affecting the healthy eggs. The dead and unfertilized eggs appeared whitish and sticky. The survival rate was determined by counting the number of fish alive in all experimental groups. Any dead fry were also removed from the aquaria. Survival rate was based on the numbers of fry that survived the heat treatment up to 24 hours post-heat treatment and expressed as percentages of the initial numbers following Angienda *et al* (2010) as follows:

$$Percent\ Survival = \frac{Final\ number\ Survived}{Initial\ number} \times 100$$

Fish growth performance was assessed in terms of Body Weight Gain (BWG), Specific Growth Rate (SGR) and Food Conversion Ratio (FCR). These growth parameters were calculated following the formulas of Hopkins (1992):

$$Body\ Weight\ Gain\ (BWG) = \frac{[(Final\ weight\ (g) - Initial\ weight\ (g)]}{Initial\ weight\ (g)}$$

$$SGR = \frac{[Ln\ (Final\ fish\ weight\ (g)) - Ln\ (Initial\ fish\ weight\ (g))]}{Time\ interval\ in\ days\ (t)} \times 100$$

$$Food\ Conversion\ Ratio\ (FCR) = \frac{Weight\ of\ dry\ feed\ fed\ to\ fish\ (g)}{[Final\ fish\ weight\ (g)] - [Initial\ fish\ weight\ (g)]}$$

3.8 Histological examination of the gonads

Histological examination of gonads was conducted at the Eldoret Pathology Diagnostics Laboratory located in Barng'etuny building in Eldoret town. Fifteen fish from each treatment were dissected by cutting ventrally, using a scalpel, from the genital papilla to the base of the pectoral fin. A window on the lateral side was opened and the viscera removed, leaving gonads in place. The anterior and posterior ligaments were cut, and gonads removed using forceps and placed in an embedding cassete then immediately dipped in buffered formalin for histological examination. The gonads were processed according to standard histological techniques described by Preece, (1972). All tissue blocks were sectioned at 8 μm using a microtome and stained with Hematoxylin and Eosin (H & E). Histological study was performed by light microscopy. Sex identification was based upon the existence of oocytes in the females and upon the lobular morphology of the testes in the males. In order to determine the effect of PSP on the structure of male and female gonads, prepared sections were viewed under the microscope to observe the structure of the seminiferous tubules with spermatocytes and ovaries with oocytes.

3.9 Statistical data analysis

Microsoft Excel 2013 was used to plot bar graphs of percent sex ratios of males and females at different larval stages, as well as a bar graph of the survival rates at different larval stages. MINITAB (version 17.0) software was used for statistical analysis to calculate means and standard error. Descriptive statistics (Mean ± SE) was used to display for distinguishing differences in growth performance parameters and proximate composition of *O. niloticus* fed on different quantities of pawpaw seeds powder.

CHAPTER FOUR

RESULTS

4.1 Sex Ratios of *Oreochromis niloticus* exposed to elevated temperature treatment at different days post-fertilization

All the fish were visually sexed by aid of a hand lens and the sex confirmed by histology. Observation of oocytes indicated female gonads hence the sample was female (Plate 4.1 a) while presence of seminiferous tubules and clusters of spermatocytes indicated male gonads hence male (Plate 4.1 b). The percentages of males and females obtained in this study at different days post-fertilization (dpf) are presented in figures 4.1 and 4.2 respectively. The results showed that the elevated-temperature treatment induced a change in sex ratio towards males although this was dependent on the days post-fertilization at which the fish were subjected to the elevated temperature treatment. The percentage of males ranged from 45.56-82.22% while the percentage of females ranged at between 17.78-54.44%. *Oreochromis niloticus* larvae subjected to the elevated temperatures at 10 dpf showed the highest percentage of males (82.22%) while the control group had the least percentage of males (45.56%). The remaining females continued to develop as females despite the elevated-temperature treatment.

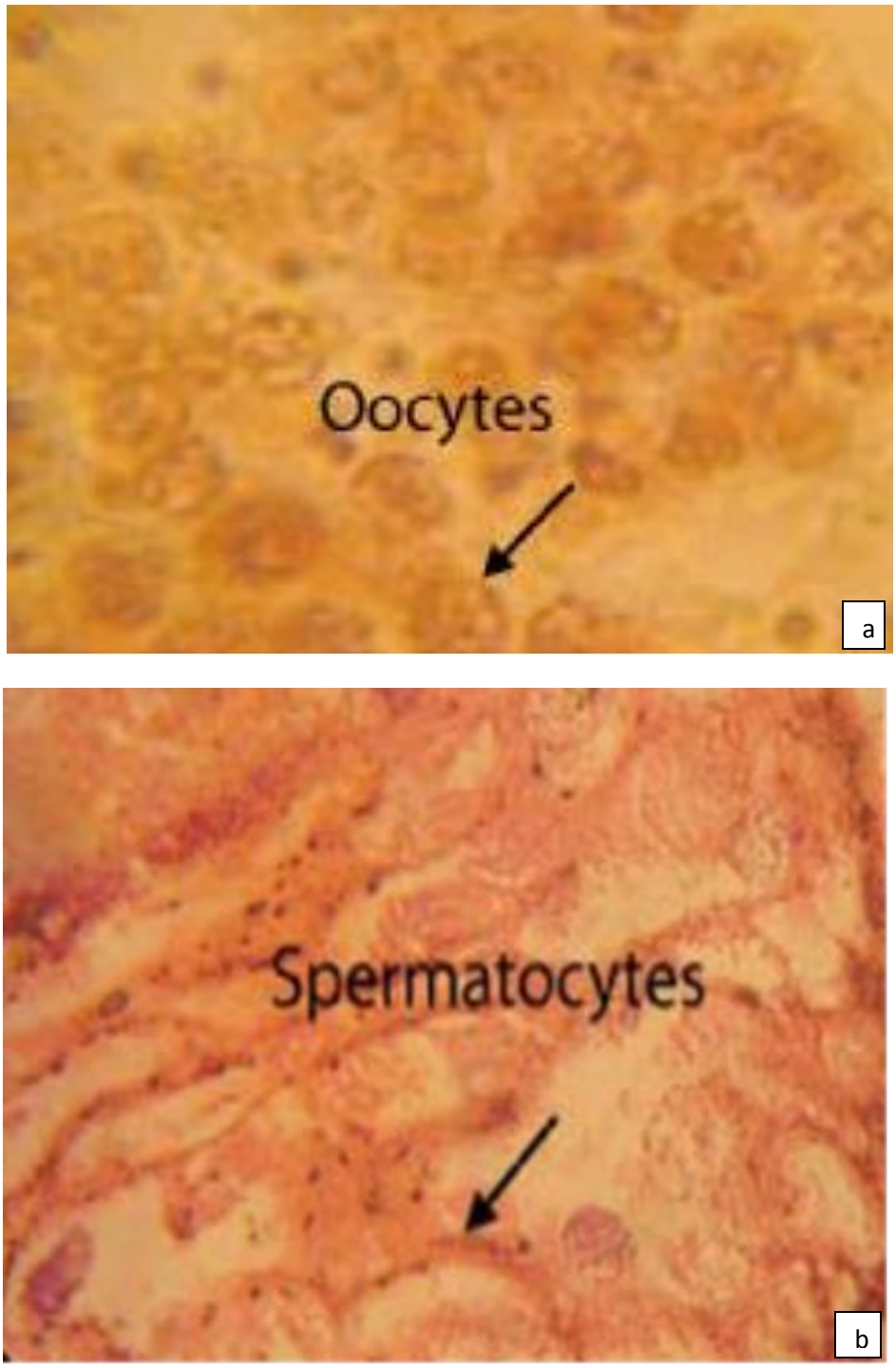


Plate 4.1: Photomicrograph of histology preparations of *O. niloticus* showing presence of oocytes in the ovaries (a - arrow), and presence of spermatocytes and seminiferous tubules in the testes (b - arrow). (Source: Author, 2017)

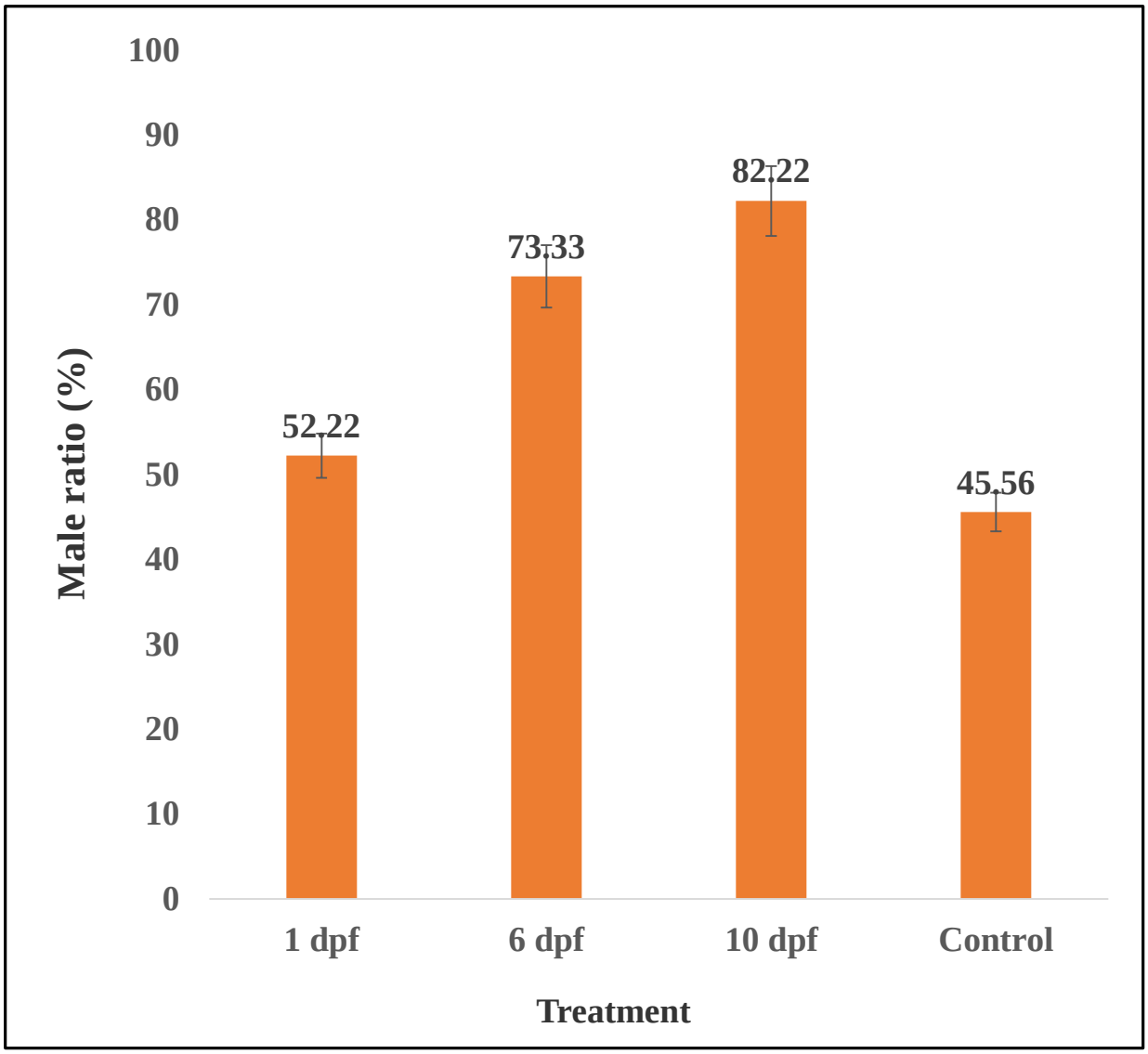


Figure 4.1: Percent sex ratios of males of *O. niloticus* subjected to elevated temperature treatment at different days post-fertilization of 1 dpf, 6 dpf, 10 dpf and control at the end of Phase 1 of the study.

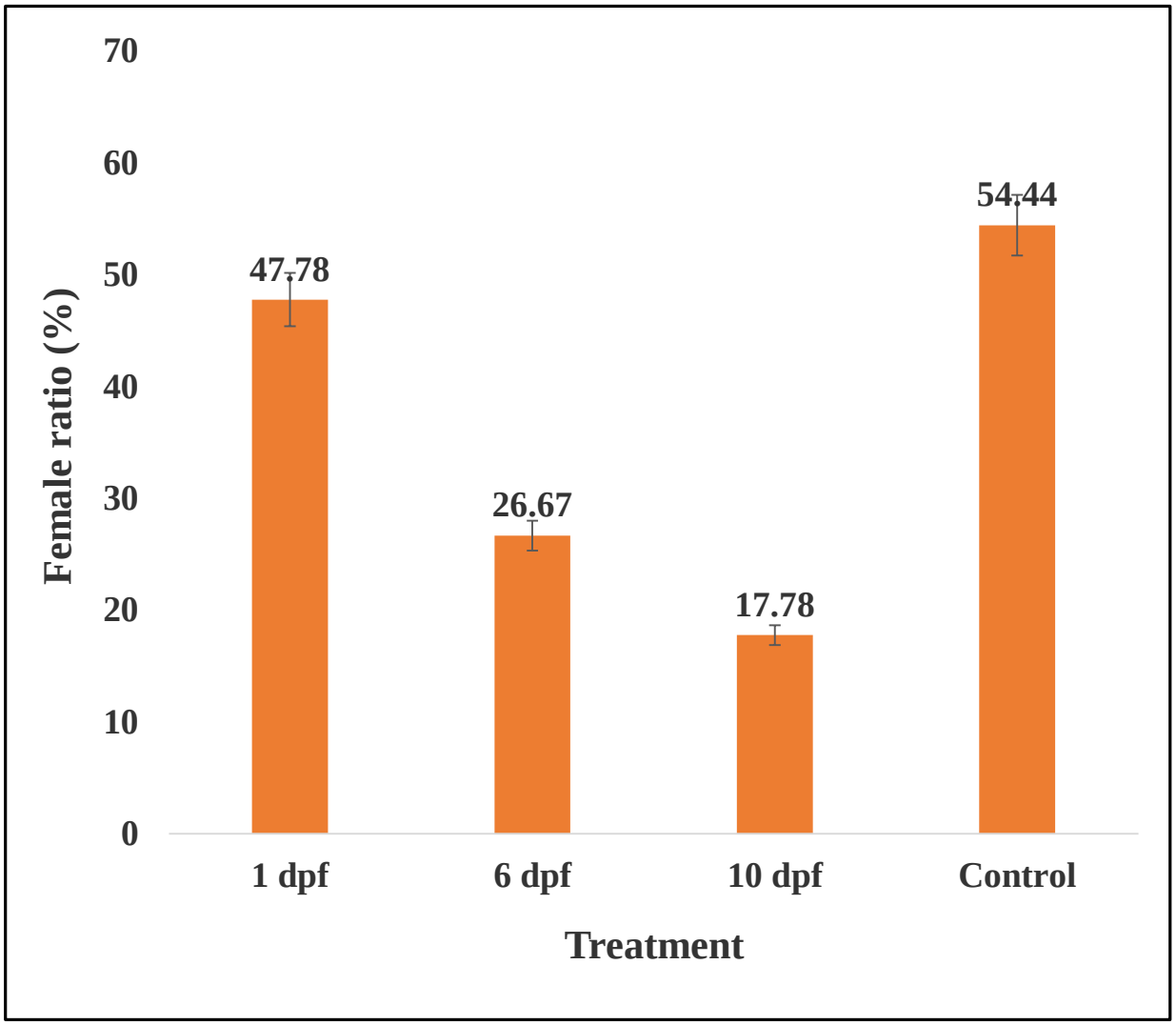


Figure 4.2: Percent sex ratios of females of *O. niloticus* subjected to elevated temperature treatment at different days post-fertilization of 1 dpf, 6 dpf, 10 dpf and control at the end of Phase 1 of the study.

4.2 Percent Survival of *O. niloticus* subjected to elevated temperature treatment

Percentage survival of *O. niloticus* in the different treatments is presented in figure 4.3. Percentage survival was generally low in all treatment groups subjected to high temperature treatment. The control group showed the highest percent survival (80.7%) while percent survival was lowest in the elevated temperature treatment of 1 dpf (43%). Percent survival at 6 dpf and 10 dpf was 68% and 73.3% respectively.

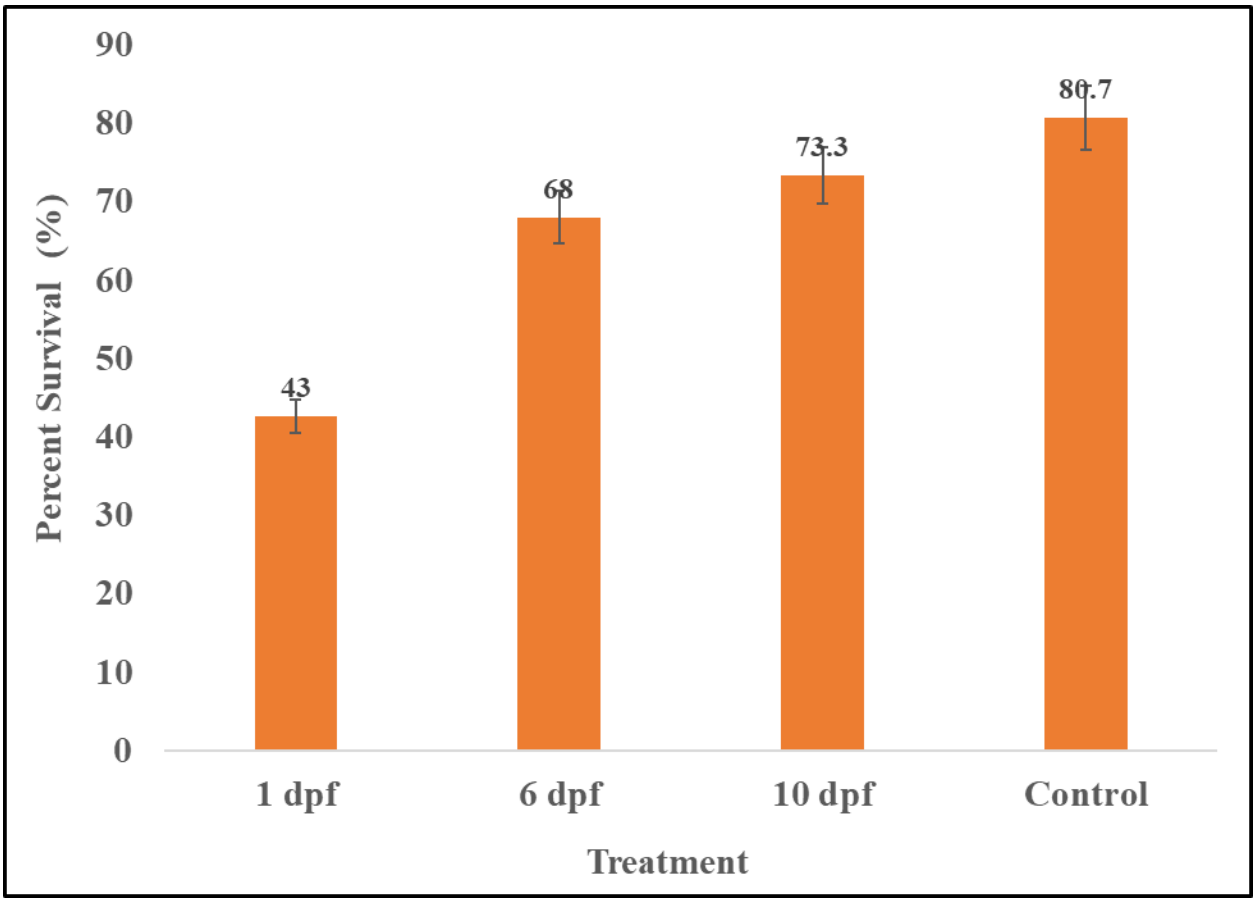


Figure 4.3: Percent Survival (%) of *O. niloticus* at different days post-fertilization during the elevated temperature treatment.

4.3 Histological structure of gonads of Nile tilapia treated with different levels of pawpaw seeds powder

Photomicrographs of histological sections of the ovaries and testes of *O. niloticus* treated with different levels of PSP are shown in plates 4.2-4.5 and histological results at different PSP levels summarized in table 4.1. The results revealed visible histological effect of feeding *O. niloticus* with PSP on the gonad structure. Ovary histology revealed normal yolk droplet and oocyte development and distribution in the treatment fed 0g PSP/kg feed. Yolk droplet, oocytes and ovary follicles were observed in the treatment fed 4g PSP/kg feed. However, this treatment showed degenerated ovary stromas. Treatment with 8g PSP/kg feed resulted in increased atretic follicles of the ovary and treatment group at the level of 12g PSP/kg feed revealed severe atretic follicles of the ovary. The ovary follicles of the 8g PSP/kg feed and 12g PSP/kg feed were degenerated. Testes histology of the 0g PSP/kg feed group showed normality with primary and secondary spermatozyte, connective tissue and normal spermatozoa. Testes of the group fed 4g PSP/kg feed had evidence of some degree of normality with presence of spermatid, spermatocytes and connective tissues. However, the testes in this treatment group had scanty spermatozoa. Treatment with PSP beyond 4g PSP/kg feeding level showed abnormalities of the testes with testes of *O. niloticus* fed 8g PSP/kg feed showing degeneration of spermatozoa in the ductus deference and those fed 12 g PSP/kg feed having deformed seminiferous lobules and severe erosion of the spermatozoa.

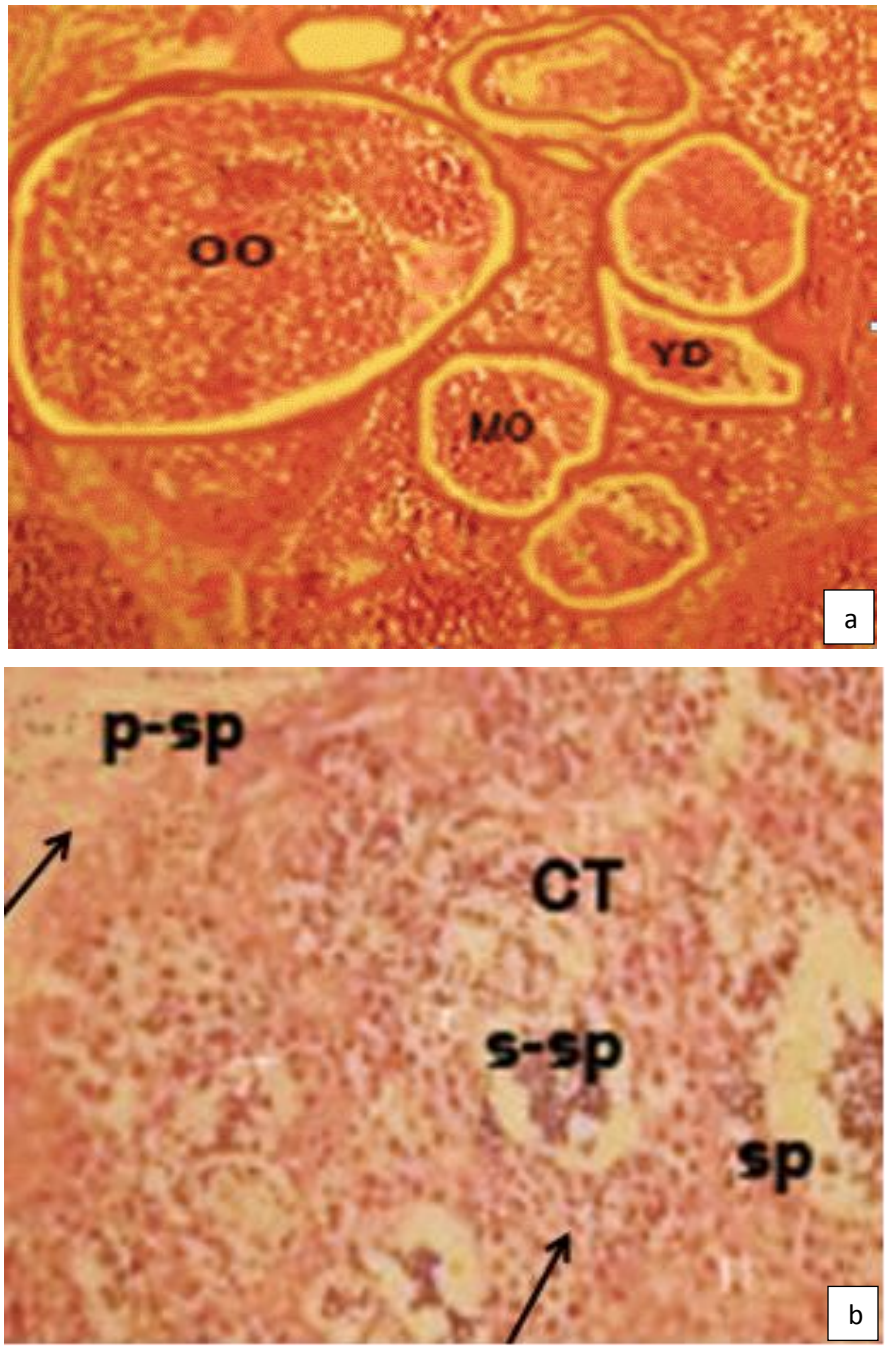
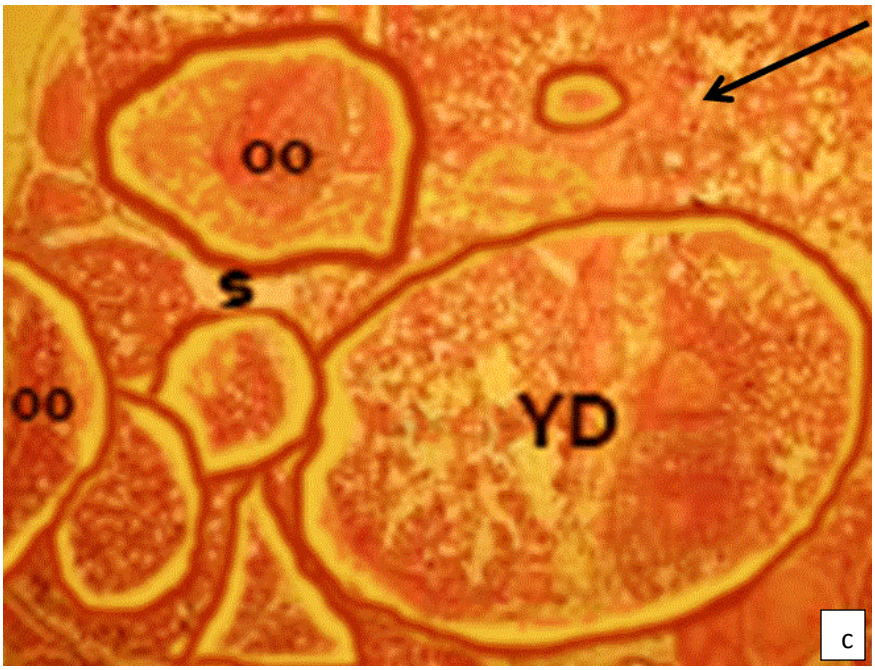


Plate 4.2: Photomicrograph of *O niloticus* ovary fed 0g PSP/kg feed showing normal oocytes (OO), yolk droplet (YD) and matured oocytes (MO) (a); and testes fed 0g PSP/kg feed showing primary spermatocytes (P-SP), secondary spermatocyte (S-SP), connective tissue (CT), and normal spermatozoa (SP) (b). (Source: Author, 2017).



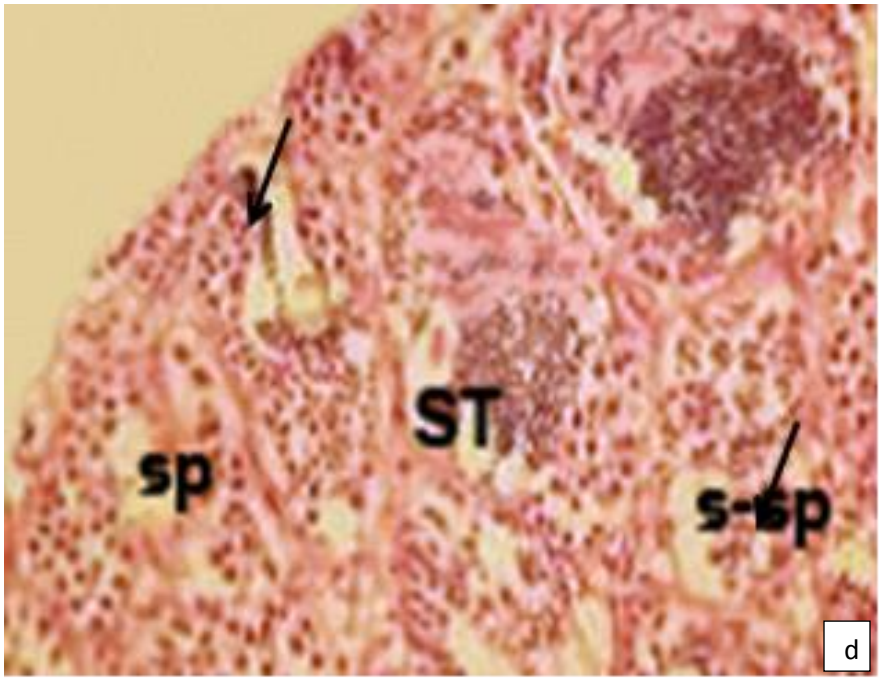
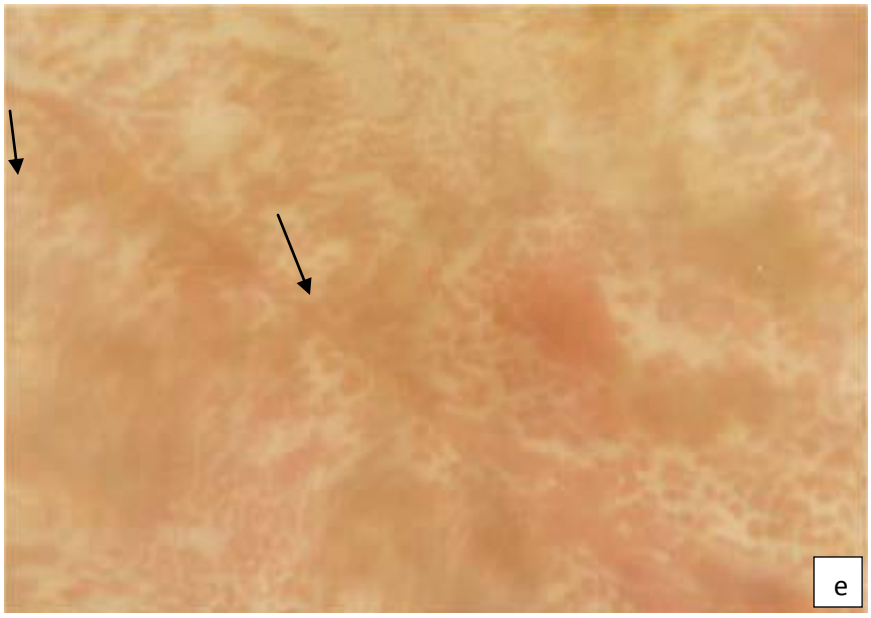


Plate 4.3: Photomicrograph of *O. niloticus* Ovary fed 4g PSP/kg feed showing matured yolk droplet (YD), developing follicle (arrow), degenerated Stroma (S) and oocyte (OO) (c); and testes fed 4g PSP/kg feed showing connective tissue (CT), spermatocytes (SP), scanty spermatozoa and spermatid (ST) (d). (Source: Author, 2017).



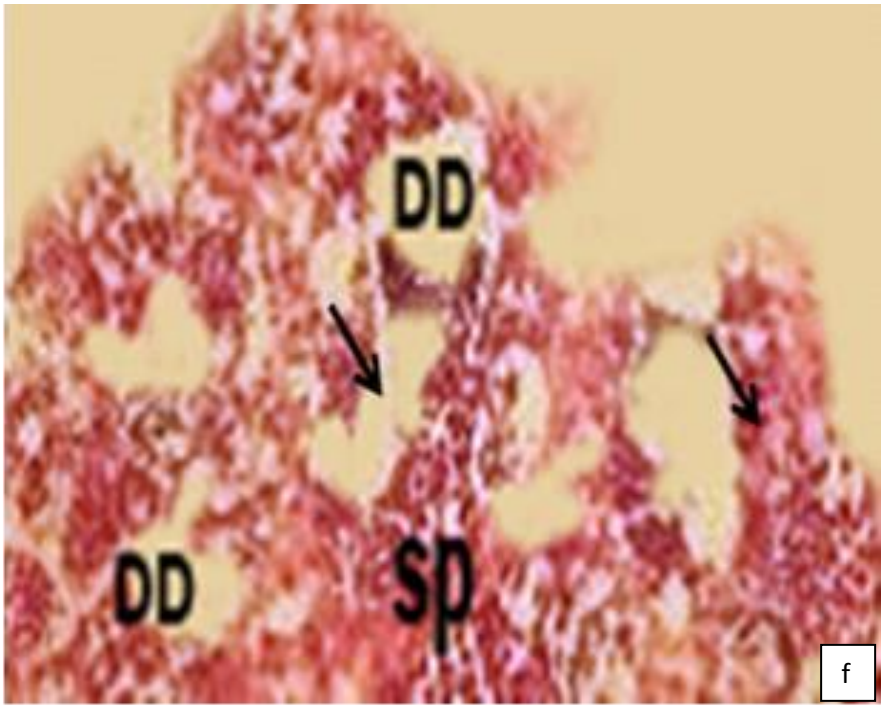
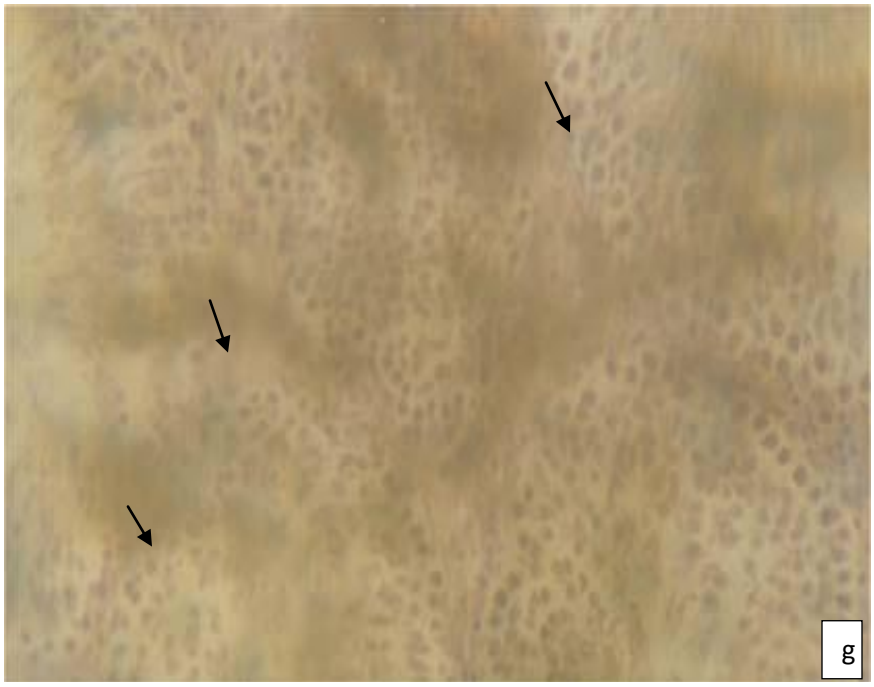


Plate 4.4: Photomicrograph of ovary of *O. niloticus* fed 8g PSP/kg feed showing increased atretic follicles (arrows) (e); and testes fed 8g PSP/kg feed showing spermatid (ST), Secondary spermatocyte (SP) and degeneration of spermatozoa in ductus deference (DD) (f). (Source: Author, 2017).



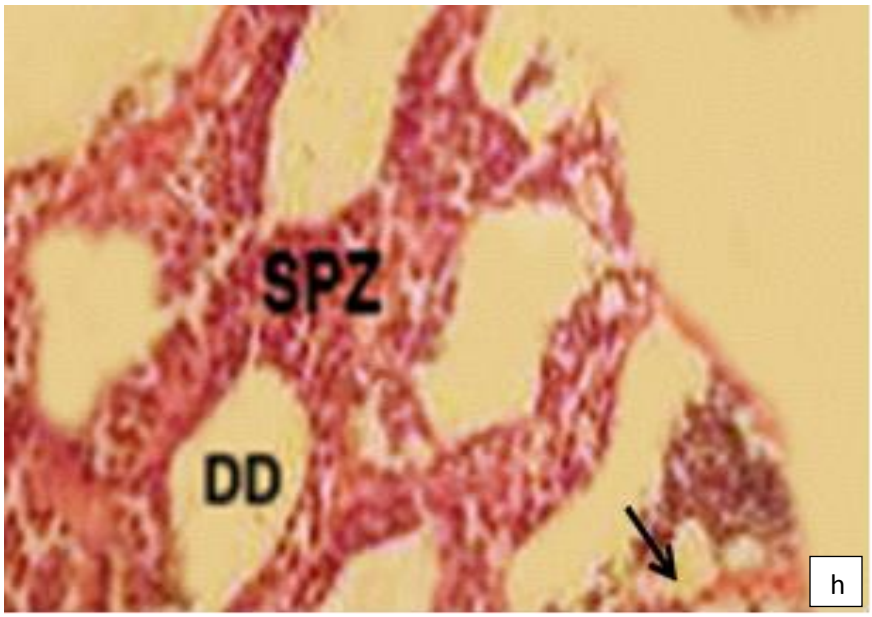


Plate 4.5: Photomicrograph of ovary of *O. niloticus* fed 12g PSP/kg feed showing severe atretic follicle (arrows) (g); and testes fed 12g PSP/kg feed showing deformation in the ductus deference (DD) and severe erosion of spermatozoa (SPZ) (h). (Source: Author, 2017).

Table 4.1: A summary of histological effect of different levels of PSP on the ovaries and testes of *O. niloticus*

Treatment	**Histological effect**	**Remarks**
0g PSP/kg feed	Ovary: Oocytes developed normally and were normally distributed. Testes: Exhibited normal development of spermatocytes and spermatozoa as well as connective tissue.	Both ovaries and testes of *O. niloticus* were normal.
4g PSP/kg feed	Ovary: Degeneration of ovary stromas Presence of oocytes, yolk droplet and developing follicle. Testes: Presence of connective tissue, spermatocytes and spermatid. Scanty spermatozoa.	Mild effect of PSP on ovaries and testes of *O. niloticus*
8g PSP/kg feed	Ovary: Moderate degeneration ovary follicles Testes: Degenerated spermatozoa in the ductus deference.	Moderate degeneration of both ovaries and testes of *O. niloticus*

12g PSP/kg feed	Ovary: Severe degeneration of ovary follicles Testes: Deformed seminiferous lobules Degenerated spermatocytes Severe erosion of spermatozoa	Severe effect of PSP on both ovaries and testes of *O. niloticus*

4.4 Proximate composition of O. niloticus fed different levels of pawpaw seeds powder

Proximate composition of *O. niloticus* fed different PSP levels at the end of the experimental period is shown in table 4.2. Crude protein content increased with increasing PSP levels upto the 8g PSP treatment level with the 8g PSP/kg feed treatment level showing the highest value (mean ±) for crude protein (53.97 ± 0.094) while the 0g PSP/kg feed had the least value for crude protein (51.25 ± 0.250). On the contrary, crude lipid content decreased with increasing levels of PSP upto the 8g PSP/kg diet, where crude lipid content was highest in the 0g PSP/kg feed (17.38 ± 1.175) and lowest in the 8g PSP/kg feed (13.9 ± 0.450). Beyond the 8g PSP/kg feed, the crude lipid level increased again as recorded in the 12g PSP/kg feed treatment group (14.75 ± 0.200). Ash content was highest in the 8g PSP/kg feed treatment level (20.05 ± 0.35) and lowest in the 0g PSP/kg feed treatment level (18.00 ± 0.250). DM was highest in the 0g PSP/kg feed (3.88 ± 0.004)

Table 4.2: Proximate composition (Mean ± SE) on dry matter basis of *O. niloticus* fed different PSP levels for 60 days

Proximate composition (%)	Treatment			
	0g PSP	**4g PSP**	**8g PSP**	**12g PSP**
Crude Protein (CP)	51.25 ± 0.25^{a}	52.88 ± 0.06^{b}	53.97 ± 0.09^{c}	53.19 ± 0.19^{c}
Crude Lipid	17.38 ± 1.18^{a}	16.18 ± 1.58^{a}	13.9 ± 0.45^{b}	14.75 ± 0.20^{c}
Ash content	18.00 ± 0.25^{a}	19.10 ± 0.64^{a}	20.05 ± 0.35^{b}	19.53 ± 0.23^{b}
Dry Matter DM)	3.88 ± 0.004^{a}	3.86 ± 0.006^{b}	3.86 ± 0.009^{c}	3.86 ± 0.014^{c}

Superscript in the same row sharing a common letter were not statistically different

4.5 Growth performance parameters of Nile tilapia fed different levels of pawpaw seeds powder

Results presented in table 4.3 showed the effect of different levels of PSP on growth performance parameters of *O. niloticus*. All growth performance parameters were statistically significant in all treatment levels (mean ± SE). The growth performance parameters were gradually improved with increasing PSP levels upto 8g PSP/kg feed, then decreased with increasing the level to 12g PSP/kg feed. Also, it could be noticed that the highest values (mean ± SE) of all growth performance parameters except for FCR compared with other levels was recorded at 8g PSP/kg diet treatment level.

Table 4.3: Growth parameters of *O. niloticus* fed different levels of PSP for 60 days

Parameters	Treatment			
	0g PSP	**4g PSP**	**8g PSP**	**12g PSP**
Initial weight (g)	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}
Final weight (g)	3.70 ± 0.05^{a}	3.61 ± 0.004^{b}	4.129 ± 0.04^{c}	3.48 ± 0.05^{d}
Average weight gain (g)	3.48 ± 0.02^{a}	3.39 ± 0.01^{b}	3.91 ± 0.03^{c}	3.26 ± 0.02^{d}
BWG	16.15	15.73	18.16	15.13
SGR (%)	4.74	4.69	4.92	4.64
FCR	2.87 ± 0.09^{a}	2.95 ± 0.02^{b}	2.56 ± 0.01^{c}	3.07 ± 0.01^{d}

Superscript in the same row sharing a common letter were not statistically different

CHAPTER FIVE

DISCUSSION

5.1 Sex Ratios of fish treated with elevated temperature at different days post fertilization

The results of this study showed the percentage of males at 1 dpf to be 52.22 % which is lower compared to male percentages of 73.33% and 82.22% at 6 dpf and 10 dpf stages respectively. This is in agreement with the findings of Rougeot *et al* (2008) who reported the pre-hatching induction of masculinization during embryonic development through temperature treatment of fertilized eggs to have relatively low success rate compared to temperature treatment post-hatching. The results of this study verified the existence of a clear genotype-by-environment (G x E) interaction during the sex determination and differentiation processes in *O. niloticus*. However, the mechanism of temperature dependent sex determination in *O. niloticus* is still not clear. There is a need to synchronize the ages at which the fry are subjected to high temperature treatment, the treatment temperatures, as well as the length of treatment. In this study, high temperature treatment was done at different larval stages and lasted for only 10 days. In the current and several previous studies (Wang and Tsai, 2000; Pavlidis *et al*; 2000), it has been shown that elevated temperatures favour masculinity in *O. niloticus* and other fishes. These results confirm that temperature is important in gonadal sex determination in tilapias. This is consistent with the findings of Baroiller and D'Cotta (2001). The results of this study indicate that morphological development towards male development in *O. niloticus* is influenced by temperature, particularly by the elevated temperature during a

restricted developmental period. This is in agreement with the observation that sexual differentiation of gonads is triggered by temperature during the critical developmental period and that exposure to elevated temperature for 10 or more days between post fertilization days 9–13 increases the proportion of male Nile tilapia (Baroiller *et al*; 1995). Apparently in the current study the treatment that yielded the highest male percentage (swim –up stage) lies within the 9-13 post-fertilization days. The current study agrees with the fact that gonadal sex is determined by temperature before the onset of gonadal differentiation in fish (Hendry *et al*; 2002).

In the interest of *O. niloticus* aquaculture development, the question that may arise from the results of this study is whether the 17.78% of female fry in the 82.22% monosex male cultures attained at swim-up stage would cause a serious over-population problem at maturity. In the tropics tilapia fingerlings reared under good conditions become sexually mature at about 4-5 months of age. They attain market sizes at 7-9 months, at which time the whole pond is harvested. This may allow the females to have only one cycle of reproduction. This situation therefore, may not present a serious overpopulation problem. Furthermore, other monosex production methods like hormone use which is widely used have also not been able to yield 100% males. This study however went further to incorporate PSP in the diet of the fish which was found to be effective in controlling the breeding of *O. niloticus*, therefore eliminating the worry of over-population in production units.

5.2 Survival rates of treatment groups subjected to elevated temperature

Elevated temperatures can induce significant larval mortality. The current study found a higher survival rate at 10 dpf compared to 6 dpf and that of 1 dpf. Survival rate is reported to be reduced if temperature increases beyond 34 ^{0}C (Azaza *et al*; 2008). Valentin and Dean (2015) reported the survival rate of *Lates calcarifer* eggs incubated at 34 ^{0}C to be 42.6% which was lower compared to other incubation temperatures of 28 ^{0}C and 32 ^{0}C. High egg mortality at 36 ^{0}C in this study probably resulted from thermal degradation of proteins that impart a direct effect on egg cell functions such as stress protein synthesis. Fish eggs consist of water, proteins and lipids, the last of which are consumed as eggs develop into Yolk sac larvae (Kamler, 2008).

In investigating the survival rate of eggs, yolk sac larvae and swim-up larvae of *Micropterus salmoides* and *Micropterus dolomieu,* Landsman *et al* (2011) found the survival of yolk sac larvae to be high during cold shocks while warm-water shocks caused significant decline. An even more pronounced decline was observed for swim-up larvae during exposure to the extreme warm-water shock. The eggs and yolk sac larvae of *M. dolomieu* and *M. salmoides* may have had greater energy reserves to fuel the accelerated metabolic rates relative to later stages (i.e. larger body sizes of swim-up larvae) that have consumed more of their lipid stores. In addition, later stages of development may consume remaining lipid reserves as their resting metabolic rates increase with body size. Their finding, however, contradicts the findings of the current study which found a higher survival rate at swim-up larvae stage compared to yolk sac larvae. This contradiction can be attributed to the fact that the two studies dealt with

different fish species. The current study nevertheless is in agreement with the previous work of Rombough (1996) which suggested that later stages of development can withstand extreme temperature better than earlier stages due to the ability of later stages to produce stress hormones at extreme temperatures thus helping to cope with the stress. More studies are however required to establish the mechanism of stress tolerance in fish, which the current study was not able to establish.

5.3 Histological structure of gonads of Nile tilapia treated with different levels of pawpaw seeds powder

The result achieved presented different levels of abnormalities in both male and female gonads of *O. niloticus* fed different levels of the pawpaw seeds powder. The dosage of pawpaw seeds powder used in the present study (0-12 g PSP/kg diet) is much lower than those administered by Ekanem and Okoronkwo (2003). In this study however, the damage done to the testes and ovaries was minimal at lower dietary PSP level of 0g PSP/kg diet and 4g PSP/kg diet, while at higher dietary PSP level of 8g PSP/kg diet and 12g PSP/kg diet, it caused disintegration of many more cells, rendering the testes and ovaries devoid of spermatids and oocytes, respectively. Degenerative stromas were observed in ovaries of females treated with 4g/kg PSP. Jegede and Fagbenro, (2008) reported necrotic ovaries in *Tilapia zilli* fed with 2g/kg Neem leaves, (*Azadirachta indica*). Comparable gonadal histological alterations were reported by Jegede, (2010) and Jegede, (2011) when *O. niloticus* were fed Hibiscus (*Rosa-sinesis*) leaves at 3g/kg and *Aloe vera* latex at 2ml/kg feed respectively. Endocrine disrupting compounds in plants that have phytoestrogens such as pawpaw seeds have been implicated to have the ability

to impair animal reproduction either by affecting gonad differentiation or by delaying maturation (Omeje, 2016). This is evident in the level of stroma degeneration in ovaries and scanty spermatozoa in testes observed in the group fed 4g/kg. Histological examination of the testes in all treatment groups except the control revealed deformation in the seminiferous tubules, degeneration in the spermatozoa and seminiferous lobule as well as severe erosion of the spermatozoa. The degree of deformities observed increased as the dosage of the PSP increased. Comparable similar results were obtained by Solomon *et al*; (2017) who reported continuous mild stroma degeneration in ovaries of juvenile *O. niloticus* fed 2g/kg; deformed seminiferous lobules and degenerated spermatozoa in the testes with increasing level of pawpaw seed meal from 2g/kg to 8g/kg. Relatively similar findings were reported by Abdelhak *et al*; 2013 who included pawpaw seeds in the basal diet of mature *O. niloticus* at 120g/kg feed. The authors further stated that the significant gonadal histological changes in high doses were irreversible while medium as well as low dosage may have reversible effects.

Omeje (2016) indicated that *C. papaya* extract contain active ingredient that can cause pronounced hypertrophy, hyperplasia and gradual degeneration of the germ cells, sertoli cells and leydig cells, as well as germinal epithelium of both gonads. However, considering the fact that in this study the PSP treatment was done in the fry stage of *O. niloticus*, the histological changes and degeneration observed was less pronounced and severe than the studies of Ekanem and Okoronkwo (2003); Ekanem and Bassey (2003); Jegede and Fagbenro (2008) and Abbas and Abbas (2011) and who studied the effect of *C. papaya* seeds on mature *O. niloticus*. This is because the exposure periods in this study coincided with the critical period of gonadal differentiation which is estimated to occur

between 7 – 28 days post hatching in tilapia (Nakamura and Takahashi, 1973). The cited literature was treated at post-juvenile stage after gonadal development had long taken place. This study however demonstrated the degenerative effect of PSP with increasing levels which may indicate that beyond an optimum level, gonads would be completely damaged and sterility could be induced. This optimum level/amount of PSP and the effect of exposure for longer periods beyond 60 days could be the focus for future research.

5.4 Proximate composition of Nile tilapia fed different levels of pawpaw seeds powder

Growth and feed conversion traits reflect the body composition of the fish. At the same time, species, genetic strain, sex and stage of reproductive cycle lead to different nutritional requirements and thereby body composition (Jauncey, 1998). The present results indicated that treatment with 8 g PSP/kg diets experimental period lead to increased crude protein (CP) and ash content in the whole body and decreased crude lipid. On the contrary, fish treated with high dose of 12gPSP/kg diet CP, but increased crude lipid in the whole body of *O. niloticus*. These results are in conflict with the findings of Abbas and Abbas (2011) who found that *O. niloticus* treated with high dose of PSP (6 g/kg/day) exhibited lowest meat quality, reflected in lower DM and CP, as well as decrease of total muscle protein and total lipids and increase in water and Ether Extract contents. Increasing water content in muscle of fish treated with PSP especially with high dose was explained by Weatherly and Gills (1987) who concluded that depletion of body constituents (protein and lipids) results in tissue hydration of inverse dynamic relationships with protein, as well as lipids and water content in the muscles.

Generally, the opposite results regarding the fish body composition may be related to fish age (fry, in feeding period in the present study), as well as nutritional and physiological status of fish. It seems that to achieve improvement in fish composition, PSP could be added to the diet very early after hatching on one side and the amount of pawpaw seeds powder could be raised to 8 g PSP/ kg diet for a long period of 60 days on the other side. The present study also identified a gap for further research work to investigate the occurrence of toxic substances in PSP.

5.5 Growth rates of Nile tilapia fed different levels of pawpaw seeds powder

Medicinal herbs and plants have recently been used as feed additives in fish due to their favourable and economically reflected results. Nowadays, most of the chemical materials are have been banned due to of health problems. This has led to concentration on the use of organic materials in fish feeds. However, the results of previous studies did not show exact positive or negative trend in most cases. The results of those studies are various factors such as the size of fish, species, duration of exposure, treatment substance exposed to and the nature of feeding. In this trend, the present results showed positive effect of PSP in the diet of *O. niloticus*. The results of the current study did not agree with the findings of Ekanem and Okoronkwo, 2003 and Abbas and Abbas, 2011 who found that addition of PSP caused decreased growth performance of *O. niloticus* during 30 day treatment period. Also, using dietary Aloe vera latex did not show significant differences in growth performance parameters and feed conversion ratio (Jegede, 2009).

Jegede (2010) reported the best overall growth response in *O. niloticus* fed the normal diet; on the other hand the weight gain, average daily weight gain and specific growth

rate were poor in fish fed the *Hibiscus Rosa-sinensis* leaf meal diets. So, the contrary trend to other studies, as well as the convulsions may be related to the different age of fish (stages), species, level of PSP and duration of exposure. While in the present study *O. niloticus* fry were fed after hatching and yolk sac absorption on different amounts of PSP (0, 4, 8 and 12 g/kg diet) for 60 days, the contrary were used in the studies of Ekanem and Okoronkwo, 2003 and Abbas and Abbas, 2011 where adult male *O. niloticus* with an average weight 40 g were fed with different levels of PSP (low dose of 4.9 g/kg/ day or high dose of 9.8 g/kg/day) for 30 days only. Similarly, Jegede and Fagbenro (2008) used different levels of PSP (0, 0.5, 1.0, 1.5 and 2 g/kg diet) for 60 days only for adult *O. niloticus* with an average weight of 40g. Generally, in addition to the effect of level and period of administration of ripe pawpaw seed extract, its extract induced variable responses according to the kind of extract, dose, duration and mode of administration (Lohiya *et al*; 2002).

In addition, the improvement in growth performance and feed utilization of *O. niloticus* in the present study may be due to the PSP content of some useful life fauna with bacteriostatic effects against several enteropathogens such as *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Osato *et al*; 1993). Moreover, the seeds of papaya have bacteriostatic activity on gram positive and negative organisms. The papaya seed macerate has a clinical potential on conjugal R plasmid transfer from *Salmonella typhimurium* to *E. coli, in vitro* and in the digestive tract of genotobiotic mice (Leite *et al*; 2005). Furthermore, *C. papaya* seeds extract contains a number of bioactive compounds (benzyisothiocyanate, alkaloids, flavinoids),

which have biological activities, e.g. anthelmintic (Kermanshai *et al*; 2001), antimicrobial, antitumor (Banerjee, 2002), and antiparasitic (Hounzangbe *et al*; 2005).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The mechanism of temperature-dependent sex determination in *O. niloticus* was not established in this study. However, according to the results of this study, temperature-induced sex reversal requires obtaining recently hatched fry at swim-up stage (immediately after yolk sac absorption) and subjecting them to elevated water temperature of 36ºC for a period of ten days. High temperature treatment has the potential as an effective and user- friendly method to Kenyan farmers for seed production in facilitating mono sex culture of all-male *O. niloticus*. Furthermore, other monosex production methods like hormone use which is widely used have also not been able to yield 100% males, therefore, high-temperature treatment that was used in this study remains highly viable for monosex production of *O. niloticus*.

It can also be concluded from the study that survival is highly dependent on the larval stage, with the 1 dpf exhibiting the lowest survival during the elevated temperature treatment period.

Histological observations of gonads in *O. niloticus* fed diets containing PSP revealed that pawpaw seeds may be effective as sterility-inducing agents as they are destructive to gonads at high PSP levels. This study gives useful information towards the determination of the contraceptive efficacies of dietary PSP in combating problems of tilapia overpopulation in ponds. PSP which are cheap and easy to obtain can be incorporated

into fish feeds with adjusted amount and be used to control breeding of tilapia fish in culture systems instead of expensive and unfavourable hormonal use.

Pawpaw seeds can be administered to *O. niloticus* in known amounts and duration in order to improve growth. However, feeding fish on pawpaw seeds results in poor flesh quality as reflected in the high ash content in the 8g PSP treatment level (20.05 ± 0.35) as well causing abnormalities in the gonads.

6.2 Recommendations

Fish farmers who wish to practice monosex culture of all male fingerlings can use elevated temperature treatment of fry at 36 ºC for ten days to high a high percentage of males.

Oreochromis niloticus eggs should be allowed to hatch at the normal temperature without the high heat treatment so as to increase their survival.

Pawpaw seeds are recommendable for use in the control of breeding in *O. niloticus*. However, pawpaw seeds powder should be supplied with known and accurate amount to fish to avoid the adverse effects which could occur due to the strong active ingredients of pawpaw seeds at high levels of administration.

Hatcheries producing seeds for cage culture in Lake Victoria need to use PSP to make the fish sterile so as to avoid genetic mix-up when the fish escape from the cages.

Farmers should incorporate pawpaw seeds to improve the growth of *O. niloticus* in the production units.

Future studies need to determine the most suitable age at which sterilization of *O. niloticus* by PSP would yield optimal results in terms of complete sterilization as well as better growth rates and flesh quality of treated fish.

Further studies need to establish the component of pawpaw seeds that is responsible for sterility and isolate it to avoid the toxicological effects of the other ingredients, as well as investigate the optimum amount and duration for administration of pawpaw seeds for optimal growth.

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