EFFECT OF PAWPAW (*CARICA PAPAYA*) SEED MEAL ON GROWTH PERFORMANCE, FEED UTILIZATION, SURVIVAL AND MASCULINIZATION OF SEXUALLY UNDIFFERENTIATED THREE SPOTTED TILAPIA (*OREOCHROMIS ANDERSONII*) FRY

A THESIS SUBMITTED IN FULFILMENT

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ABSTRACT

The objective of this study was to determine the effects of dietary *Carica papaya* seed meal supplementation at different levels on growth performance, feed utilization, survival rate and sex ratio of sexually undifferentiated three spotted tilapia (*Oreochromis andersonii*) fry. Six treatments (replicated thrice) were designed based on the control (basal diet), 17 α-methyltestosterone (MT) (60 mg) and *C. papaya* seed meal incorporated into tilapia feed at 5, 15, 25 and 35 g / kg diet and administered to freshly hatched fry (9 days old) for 120 days. The swim up fry (initial body weight 0.04 g / fry) were collected from the aquarium and randomly distributed ($n = 80$) at each of eighteen black bins with a holding capacity of 200 L. The results showed that the growth performance of the fish fed varying inclusion levels of pawpaw seed meal did not differ significantly from those fed basal diet and 60 mg of MT. A decrease in growth performance and poor feed utilization capacity was observed in the treated fish with increasing dietary *C. papaya* inclusion levels from 15 g / kg to 35 g / kg diet, suggesting that it had inhibited the growth at high dosage. Pawpaw seed meal was able to skew the sex ratio in favour of males, from the expected ratio of 1:1 male: female for all the treatments. The percentage of males increased for the treatments with increasing the dietary inclusion level, with the highest masculinization percentage (82%) ($P < 0.05$) observed in fish fed with 15 g and 35 g of *C. papaya* extracts / kg diet. Dietary *C. papaya* did not significantly change the fish survival rate among the treatments ($P > 0.05$), signifying that it had no lethal effect. This study showed the possibility of using pawpaw seed meal as a natural agent to induce sex reversal in three spotted tilapia. Due to the safety, ease of biodegradation and local availability of this naturally sourced sex reversal agent, the study recommends that, it could be used as a viable alternative to popular synthetic

hormones. In order to improve growth and induce sex reversal at the same time, the study recommends the use of 5 g but not ≥ 15 g *C. papaya* extracts / kg diet to be economically viable and efficient for fish farms.

Keywords: *Carica papaya,* Aquaculture, Growth, Reproduction, *Oreochromis andersonii*, Phytochemicals, Sex reversal

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Iipinge LN, Gabriel N, Iitembu J, Omoregie E, Shimooshili K. 2018. Effect of Pawpaw (*Carica papaya*) seed powder on growth performance and masculinization of sexually undifferentiated three spotted tilapia (*Oreochromis andersonii*). African Journal of Aquatic Science. Under Review.

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LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization
USA	United States of America
FW	Final Weight
WG	Weight gain
ARG	Absolute growth rate
SGR	Specific growth rate
VSI	Viscerosomatic index
HIS	Hepatosomatic index
GSI	Gonadosomatic index
CF	Condition factors
FI	Feed intake
FCR	Feed conversion ratio
FER	Feed efficiency ratio
PER	Protein efficiency ratio
DO	Dissolved oxygen
pH	Potential hydrogen
FOSS	Free and Open Source Software
ANOVA	Analysis of Variance
SPSS	Statistically Package for Social Sciences

MT	Methyltestosterone
PSM	Pawpaw Seed Meal
SANUMARC	Sam Nujoma Marine and Coastal Research Centre

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DEDICATION

I dedicate this thesis to my parents that matter most in my life:

My mother Miss. Helena Amukoto and

My father Mr. Andreas Iipinge

DECLARATION

I Linda Nuushona Iipinge hereby declare that this study is my own original work and it is a true reflection of my research and that this work or any part thereof has not been submitted for any degree at any other institution.

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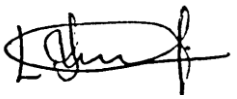
Linda Iipinge		08 APRIL 2019
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CHAPTER 1

1. INTRODUCTION

1.1 Background of the study

Tilapia (including three spotted tilapia, which are unique in Namibia) are freshwater fish which are most important in aquaculture and served as major source of protein in many developing countries for instance Sub-Saharan Africa in 2002 (FAO 2002). This group of fish is widely cultured in many parts of the world because it displays many desired characteristics including high growth rates, the ability to spawn in captivity and acceptance of natural and artificial feed, efficient food conversion, adaptability to a wide range of environmental conditions and resistance to diseases under cultured conditions (Phelps and Popma 2000; El-Sayed 2006). Despite the aforementioned characteristics, tilapia cultures are however, negatively affected by the onset of precocious sexual maturity and prolific breeding, which in turn results in overpopulation and consequently stunted growth, production of uneven fish sizes, longer culture periods as well as low marketable sized fish (Varadaraj and Pandian 1990; Popma and Masser 1999; Toguyeni et al. 2002).

The concept of monosex culture production of tilapia (preferably male) presents an opportunity to control undesirable spawning, which minimizes stunted growth, shortens culture duration, and subsequently improves yield and economic return (Omeje 2016). All - male cultures of tilapia are preferred because they exhibit rapid growth (Megbowon and Fashina - Bombatta 2010; Beardmore et al. 2001), as they spend less energy on reproduction (Popma and Masser 1999) compared to females. Frequent breeding behaviour and precocious maturity of tilapia have enthused the development of various techniques including hand sorting of sexes (Guerrero 1982), hybridization (Hickling 1960), genetic manipulation (Pandian and Varadaraj 1988;

Mair et al.1991; Liu 2013), temperature (Desprez and Melard 1998; Azaza et al. 2008; Khater et al. 2017), and hormonal induction (Nakamura and Takahashi 1975; Nakamura 1975; Tayamen and Shelton 1978; Goudi et al. 1983; Jae-Yoon et al. 1988; Ferdous and Ali 2011; Kefi et al. 2012; Jensi et al. 2016), to control spawning in order to achieve an all-male population with good marketable sizes (Beardmore et al. 2001; Abad et al. 2007).

The synthetic sex reversal hormone (17 α methyltestosterone) is considered to be the most effective method of producing an all-male tilapia population and is widely used because it saves time and produces effective results (Phelps and Popma 2000). Synthetic hormones exert their effects once incorporated in the diet which is administered to sexually undifferentiated fry at about 2 weeks after hatching (Fuentes-Silva et al. 2013) in order to achieve the phenotypic sex reversal (Baras et al. 2000). The fry fed sex reversal hormone incorporated diet at an appropriate dosage and period, will be converted from genetic females to phenotypic males (Johnstone and Youngson 1984; Ridha and Lone 1990; Pandian and Sheela 1995). Despite its effectiveness, synthetic sex reversal hormones had been considered to be carcinogenic (Velazquez and Alter 2004) and reported to have effect on tilapia culturists when they come in contact with the hormone during feeding of fish in 2006 (FAO 2006; Guerrero 2008) and environment when effluent is discharge into the environment (Heberer 2002). Consequently, its use has been prohibited in some countries for example EU countries and India (White et al. 2006). The concerns about the expensive nature and health implications of steroid hormones in food fish production and difficulty in obtaining such hormones have inspired to search for alternative methods. The World Health Organization also encourages utilizing of medicinal herbs and plants to substitute the use of steroid hormones through global

trend to go back to natural substances, which are less expensive, environmentally friendly, locally available and biodegradable for commercial aquaculture use (Farrag et al. 2013). Pawpaw *(Carica papaya)* is one of the plants, which contains phytochemicals that have attracted research in this regard (Ampofo-Yeboah 2013). Pawpaw seed meal (PSM) contain active ingredients such as caricain, carpasemine enzyme (a plant growth inhibitor) and oleanolic glycoside, which had been reported to cause sterility in male rats (Kobayashi et al. 2008) and used to control prolific breeding of *O. niloticus* (Ekanem and Okonkwo 2003; Ayotunde and Ofem 2008) by reversing the sex of fish in favour of males. A study by Ampofo-Yeboah (2013) on the potential use of pawpaw seed meal when incorporated into fish feed, produced 65% masculinization rate in *O. mossambicus*. Phytochemicals exert their effects by blocking the receptor sites against the oestrogen (Younes and Honma 2011; Rietjens et al. 2013), or mimicing the action of oestrogen that control sexual differentiation and gonad development of fish (Ribeiro et al. 2012; Ampofo-Yeboah 2013). In addition to dietary pawpaw seed meal being a potential tilapia reproduction control agent, it 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. In addition, the information on the use of pawpaw seed meal incorporated in the basal diet to reverse the sex of the fish will be able to provide treatment procedures for tilapia precocious breeding and further assist fish farmers to enhance their economic returns by farming

profitably with tilapia as well as reduce or eliminate the utilization of unsustainable synthetic drugs. However, before pawpaw seed meal is commercialized and made available for usage, comprehensive research on its performance in different farmed species is necessary.

1.2 Statement of the problem

The culturing of an all-male tilapia stocks, which generate better yield, offers a solution to the problems associated with overbreeding such as overcrowding leading to stunted growth and uneven size of fish. Methyltestosterone hormones and other pharmaceutical drugs, for instance antibodies which are commonly used to alter the sex ratio and promote growth in fish, were discovered to have negative impacts on the environment, human and fish. Several studies revealed that herbal extracts such as *C. papaya* (pawpaw) seeds have been reported to improve growth and cause masculinisation in fish. Although they display those useful properties, studies on their application in aquaculture, especially in *O. andersonii* (three spotted tilapia) and other tilapia species in general, are limited. Their effects on the sex ratio (Ampofo-Yeboah 2013; Omeje 2016), histology of gonads (Ekanem and Okoronkwo 2003; Jegede and Fabrenro 2008) as well as growth performance and feed utilization (Farrag 2013) were only reported in *O. mossanbicus.* More knowledge is therefore required to evaluate the effectiveness of dietary *C. papaya* seeds in *O. andersonii* fry as a sex reversal agent, growth promoter and feed digestive enhancer. This study therefore aimed to address this gap in knowledge.

1.3 Aims of the study

The study aimed to investigate the potential of pawpaw seed powder as a growth promoter and sex reversal agent in *O. andersonii* (three spotted tilapia). The specific objectives were:

1. To assess the effects of dietary *C. papaya* seed meal on growth performance indexes i.e. final body weight (FW), weight gain (WG), absolute growth rate (AGR), specific growth rate (SGR), viscerosomatic index (VSI), hepatosomatic index (HIS), gonadosomatic index (GSI) and condition factors (CF), feed utilization (feed intake, feed conversion ratio, feed efficiency ratio and protein efficiency ratio) and survival rate in *O. andersonii*.
2. To investigate the potential effects of dietary *C. papaya* seed meal on sex ratio of sexually undifferentiated *O. andersonii*.

1.4 Hypotheses of the study

The study hypothesised that:

1. H_0 = Feeding sexually undifferentiated *O. andersonii* fry with dietary *C. papaya* seed powder for 120 days does not enhance their feed utilization capacity and ultimately improve their growth performance and survival rate.
2. H_0 = Feeding sexually undifferentiated *O. andersonii* fry with different dosages of dietary *C. papaya* seed powder for 120 days does not affect their sexual differentiation by altering their sex ratio toward male populations.

1.5 Significance of the study

Previous studies have discovered that the pawpaw plant is an important plant as it can be used as an antimicrobial agent, growth promoter, antioxidants, sex reversal,

and immune-stimulants as well as used as a medicine to treat other several health conditions. Pawpaw seeds are believed to possess phytochemicals that enhance the digestive systems, feed utilization capacity and ultimately improve the growth performance of the fish. In addition to medicinal traits, phytochemicals were reported to alter sex ratio through blocking the biosynthesis and action of estrogenic by inhibition of aromatase activity (Wang 2002) or bind to the receptor sites against the oestrogen, that could functionally mimic the action of oestrogen which control sexual differentiation and gonad development of fish (Ribeiro et al. 2012). The present study investigated the effectiveness of pawpaw seed powder as a feed additive for *O. andersonii*, extending previous works on pawpaw in tilapia and contributing to the existing information on the use of medicinal herbs in aquaculture.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Current status of aquaculture in the world

Aquaculture refers to rearing, breeding and harvesting of fish, shellfish, plants, algae and other organisms in all types of water environment. Aquaculture is considered as the fastest food producing sectors in the world, which accounted for about 50% of the world food fish production in 2014 (FAO 2014) and projected to contribute 41% of the world of fish production by 2020 (Krishen et al. 2009). The contribution of aquaculture to the total global fisheries landings was very trivial during 1950 – 1970 (ranging from 3.2% in 1950 to 5.2% in 1970) however, it continued to thrive in 1980 - 1990 from 9.6% to 16.3%, respectively (El-Sayed 2006). In addition, aquaculture production was reported to have thrived (in the 1990s and early 2000s) to an outstanding rate, reaching an annual rate of 32.1% in 2000 and 35.2% in 2001 (El-Sayed 2006) and creating a wider gap between capture fisheries and aquaculture production. In support of this, Tacon (2003) reported that the average annual compound growth rate of aquaculture production was 9% per year during 1970 – 2000, compared with only 1.3% for capture fisheries. On the other hand, FAO (2002; 2003) reported the aquaculture growth to have a growth rate of 11 percent annually in comparison to terrestrial farmed meat production and the 1.4 percent of stagnating capture fisheries. Half of total global aquaculture production in 2002 was finfish (El-Sayed 2006). Aquaculture production is predicted to expand further and contribute to meeting global fish demand, the acquisition of food security and income generating activity (Ampofo-Yeboah 2013).

2.2 Tilapia as a source of food

Tilapia is a common name for certain species of fishes belonging to the family Cichlidae, represented by numerous freshwater species. Tilapias are native to Africa but have been introduced in numerous tropic and subtropics regions (for instance China, Middle East and South America) because of their adaptability to various environmental conditions (Popma and Masser 1999; Omeje 2016). The low cost of production, it is acceptability by consumers as a food fish and its high economic value, promote its culture worldwide (Siddiqui and Al-harbi 1995; De la Fuente et al. 1999; Rad et al. 2006; Shallof and Salama 2008; El-Kashief et al. 2013). Nile tilapia is a second most important group of freshwater fish in aquaculture in 2010 (FAO, 2010) with an improved production from 2, 5 million tons in 2007 to 3.6 million tonnes in 2008 globally, an increase of 1.1 million tonnes over a year in 2012 (Megbowon 2011, FAO 2012).

Tilapia species serve as an important food source in most parts of the world and considered as a significant source of easily digested Omega 3 fatty acids, vitamins (especially A, D, E and B - complex) and minerals (including calcium, iodine, zinc, iron and selenium) in 2016 (Bondad-Reantaso et al. 2005; FAO 2016). Tilapia accounted for about 25 percent of the global population intake of animal protein and is considered as an important food source worldwide especially in the low-income food deficit developing regions like Sub-Saharan Africa with the species in the genus *Oreochromis* in 2011 (FAO 2011).

2.3 Biology of tilapia

2.3.1 Taxonomy

The genus of *Oreochromis* belongs to the family Cichlidae, which can be distinguished from other families of bony fishes by the presence of a single nostril on either side of the snout along with heavily spines on their dorsal fins as well as an interrupted lateral line running superior along the interior part of the fish and inferior along the posterior portion (Popma and Lovshin 1996; Shelton and Popma 2006). Cichlids are native to Africa, whereby more than 70 species have been identified (Philippart and Rewet 1982; Macintosh and Little 1995; MacAndrew 2000), but only few species are of commercial significance (Popma and Lovshin 1996).

Commercially, important species are currently mainly the three genera namely *Oreochromis*, *Tilapia* and *Sarotherodon*, which can be distinguished based largely on their reproductive and developmental characters, biogeography, feeding as well as structural characteristics (Trewaves 1982). Previously, all commercially important tilapia were grouped together under the genus *Tilapia* (Lowe 1959), but recently, two other groups were established based on the parental care investment of the particular species (Omeje 2016). The three genera are all nest builders and substrate spawners but differ in brooding of eggs as well as fry. In contrast with brooding of eggs and fry, in the tilapia species (*T. zilli* and *T. rendalli)*, both males and females guard the fertilized eggs and hatched larvae in the nest until they become independent; in the *Sarotherodon* (*S. galilaeus*) both males and females incubate the fertilized eggs and fry orally; and in the *Oreochromis* species (*O. andersonii*, *O. mossambicus* and *O. niloticus*) oral incubation of both fertilized eggs and fry is only done by females.

2.4 Fecundity and egg characteristics

Fecundity in most tilapia varies inversely with the parental care exhibited by species (Omeje 2016), among fish of the same species as well as female of similar sizes especially in large fish classes (Coward and Bromage 1999; El-Sayed 2006). Mouth brooding species (including *O. andersonii*, *O. niloticus* and *O. mossanbicus*) that exhibit higher levels of parental care are characterized by low fecundity and large egg sizes while substrate spawning species (for instance *Tilapia zillii* and *Tilapia mariae*) that do not exhibit parental care have high fecundity and small egg sizes (Coward and Bromage 2000; Jegede and Fawole 2011). The fecundity of mouth brooder for instance *O. andersonii* can be 790-1800 eggs per spawn (Kefi et al. 2012) but can exceed 12 000 in *Tilapia zillii* (Coward and Bromage 2000). Substrate spawners produce small sized eggs ranged from 1.14 - 2.29 mm (Jegede and Fawole 2011) compared to mouth brooders which produce large sized eggs. For example, the eggs sized produced by *O. andersonii* can be ranged from 0.1 -3.40 mm (Kefi et al. 2010), while *O. niloticus* can produce the egg size ranged from 2.1-7.90 mm (De Graaf et al. 1999; Pena- Mendoza et al. 2005). In addition, fecundity and egg size in tilapia were observed to be significantly correlated to the length and age of the fish, but with high variability (Rana 1986; Little 1989; Ridha and Cruz 1989; El- Sayed 2006). Little (1989) and El-Sayed (2006) discovered that large brood stocks of *O. niloticus*, which frequently oviposit more eggs with longer spawning interval (105-116 days) tend to produce more eggs per clutch compared to the small brood stocks which produce many more eggs with shorter spawning interval. Equally important, Rana and Macintosh (1988) revealed that large eggs contain more yolk compared to small eggs, which often lead to larger fry with enhanced growth and high resistances to diseases and severe environmental condition. However, the decision on the type of

brood stocks to use for culture depends on the hatchery managers based on the pre-set objectives and targeted outputs (El- Sayed 2006).

2.5 Mode of reproduction

The reproduction mode of these species is relatively enthralling, as they display an elaborated courtship behaviour (Rana 1990; El-Sayed 2006). Breeding initiate with the male fish which creates a fiercely guarded spawning nest at the bottom of the pond, followed by the courtship between the resident male and a visiting female, which only lasts for a few minutes (Rana 1990; El-Sayed 2006). After a short mating ritual, the female lay a batch of eggs into the nest, which are then fertilized with a cloudy milky milt released onto them by the male (El- Sayed 2006). After the fertilization process, the female returns immediately and pick up the batch of fertilized eggs into her buccal cavity, hold and incubate them throughout until they hatch and yolk sac is fully absorbed in 2012 (Popma and Lovshin 1995; Popma and Masser 1999; El-Sayed 2006; FAO 2012), with the brooding lasts for 20-22 days (Oliveira and Almada 1996). As the swim up fry start to mature, they start to leave the female's mouth in which they were guarded from predators and on any sign of danger the female will often offer refuge for the fry in her mouth. Whenever the fry are released for feeding, they always swim in schools with their mother until they reach the size which they can feed for themselves. The feeding of female is however, interrupted during the brooding period (Specker and Kishida 2000), thus it is worthwhile to remove the eggs or fry from their mouth during incubation period to enhance their feed diet frequency.

2.6. Size at maturity

Sexual maturity in fish it is determined by the environmental conditions, age, size and it varies considerately between species (Macintosh and Little 1995). Generally, fish species used in aquaculture will not reproduce in the culture environment before reaching marketable size (100 – 200 g), however, it is different in tilapia species as they mature and reproduce at an early age. Moreover, under favourable conditions most tilapia fish (for instance *O. andersonii* and *O. mossanbicus*) reaches maturity within 3 months at the size less than 100 g (Phelps and Popma 2000), but if the breeding is not controlled, brooders will continue to reproduce and offspring will compete with original stock for food resulting in stunted growth and unmarketable fish (Popma and Lovshin 1995). In addition to sexually maturity, Nile tilapia was reported to reach their sexual maturity within 6 – 8 months at the size 30 g to 50 g under favourable aquaculture conditions (Siraj et al. 1983) and if the spawning is not regulated, will results in production of irregular sizes and competition for space with the original stock. Despite an increase of seed production in aquaculture, Schreibman et al. (1989) revealed that early maturity fish contain small sizes compared to late maturing fish due to slow growth rate during reproduction stage, consequently, male fish gender is preferred for culture. In addition, studies reported that the weight of tilapia fish increases with increasing dietary protein (De Silva and Radampola 1990; El-Sayed et al. 2003), thus, it is significant to consider feeding regime, quantity and quality of the feed in order to increase the productivity of the brooders as well as their sizes at first maturity.

2.6 External factors affecting growth and reproduction in tilapia fish

2.6.1 Water temperature

Temperature is one of the most commonly studied environmental factors that affect the physiology, growth, reproduction and metabolism of tilapia (El-Sayed 2006). Each fish species has an optimum temperature for growth and survival (Brett 1979; Gadomski and Caddle 1991), which may change with age and size as juveniles of many species prefer warm water than adult (McCauley and Huggins 1979; Pedersen and Jobling 1989). For instance, three spotted tilapia and Mozambique Tilapia grow best at 28.89°C during larval stage and then at 27.78°C during fry stage, while juvenile to adult prefer 25.56°C and 27.78°C respectively (El- Sayed 2006).

In general, tilapia species are thermophilic and known to tolerate a wide range of water temperature (El-Sayed 2006). Temperature with an optimum range of about 25- 30°C is considered as ideal for normal growth, reproduction and development of tilapia (Balarin and Haller 1982; Chervinski 1982; Philippart and Ruwet 1982; El-Sayed 2006), but may also tolerate low temperature regimes with a range of about 7-10°C for a short period of time (Balarin and Haller 1982; Chervinski 1982; Jennings 1991; Sifa et al. 2002; El-Sayed 2006). Although tilapia tolerates low temperature, longer exposure will certainly lead to reduced growth rate, deprived feed conversion ratio, increased susceptibility to infections, and high mortality (El-Sayed 2006). Severe mortality is determined to occur at the temperature of about 12°C while the feeding is reduced below 20°C and inhibited at 16°C (Balarin and Haller 1982; Chervinski 1982; El- Sayed 2006).

Additionally, the temperature above 40°C - 42°C is considered critical to most tilapia as it triggers them to be more susceptible to low dissolve oxygen conditions (Chervinski 1982; Philippart and Ruwet 1982). A sudden change in temperature

during water exchange is precarious to fish as it stresses the juveniles, causing weakened immune systems and even upset the balance of established biological colonies in 2012 (FAO 2012). Thus, it is important to make sure that water that will be added to tilapia pond or tank have the same temperature as the water the fish were already accustomed or there is a slight difference to inhibit fish from the shocking in 2012 (FAO 2012). Apart from that, increased temperature plays a major role in the initiation of gonadal maturation and eggs development in tilapia (Omeje 2016). Seasonal spawning in most tilapia is subjected to virtuous elevated temperature regimes which significantly influence both oocytes development and gonad-somatic index (Omeje 20016). Reproduction is determined to occur best at temperatures higher than 26.7°C and does not occur below 20°C (Aureli and Torrans 1988; Towers 2005), as the gonads and reproductive cycles tend to be retrogressed and interrupted at that level (Madu 1989). Popma and Lovshin (1996) reported that gonadal tissue in *Oreochromis spp*. starts to differentiate into ovarian tissue at an average water temperature of 24°C to 28°C at the size and age of 11 to 14 mm and 3 to 4 weeks after hatching.

2.6.2 Dissolved oxygen

Dissolve oxygen is one of the major environmental factors that determine metabolism, feeding, growth and reproduction in fish (El-Sayed 2006). DO fluctuations is affected by photosynthesis, diel fluctuation and respiration, which must be fully considered for enhanced performance of fish (El-Sayed 2006). Tilapia grow well when the aeration in the tank keep DO level above 2.0- 2.5 mg / L as well as avert morning dissolve oxygen concentration from falling below 0.7 – 0.8 mg / L

in 2012 (FAO 2012). Tilapia are known to tolerate very low DO concentrations (0.1-0.5 ml / g) as well as conditions of high oxygen supersaturating (up to 400) which occasioned by high photosynthesis resulting from phytoplankton and macrophytes blooming only for a short period of time (Morgan 1972). Prolong exposure (DO below 1 mg / L) will lead to reduced metabolism, growth, disease resistance as well as causes regression and interruption cycles in fish (Madu 1989). The level of DO in water is known to be reduced by cumulative water temperature, which increases respiration rate and oxygen consumption leading to high rate of metabolism and increased tissue demand for oxygen (El-Sayed 2006). Studies reported that the rate of oxygen consumption in tilapia increased from 0.78 to 0.97 mg / L / h with increasing water temperature from 37°C to 42°C (Becker and Fishelson 1986; Franklin et al. 1995). On the other hand, Job (1969) indicated that the respiration of tilapia was independent of DO at the oxygen saturation of 25 – 32% at the temperature ranging from 15°C to 30°C while below these saturation levels the metabolic rate became dependent on the oxygen available and mortality occurred when DO remained below 20% saturation for more than 2-3 days. Apart from that, handling stress was also discovered to have a significant effect on oxygen consumption in tilapia. This was reported by Ross and Ross (1983) who discovered that handling stress in Nile Tilapia increased oxygen consumption rate from 150% to 300% of the resting value and did not return in many cases to the resting value after 3 hours. Therefore, the author suggested the fish to be returned to water containing high levels of DO and not to be fed for at least an hour.

2.6.3 pH

The present of bicarbonate and carbonate anions in the water determines the buffer system of pH (De Holanda Cavalcante et al. 2010). In general, tilapia can survive in pH ranging from 5-10 but they grow and produce well in the pH range between 7-8 (El-Sherif and El- Feky 2009). The lower and upper lethal levels of pH for tilapia (4 and 11) (Bhujel 2000) were further reported to cause behaviour changes, damage of gill epithelial cells, reduction in the efficiency of nitrogenous excretion and increased mortality (El-Sayed 2006). In support, fingerling and adult Nile Tilapia exposed to pH 2-3 were reported to show rapid swimming and opercula movements, surfacing and gulping of air, lack of body position and mass mortality with 1-3 days (Wangead et al. 1988). Similarly, Chen et al. (2001) discovered that *O. mossambicus* exposed to high pH for 7 days decreased ammonia excretion but increased urea nitrogen excretion. Although there are few literatures on the influence of pH on the reproduction process in fish (Omeje 2016), a higher proportion of male was reported among Apisogamma species at the pH level of 4.5 (Romer and Beisenherz 1996).

2.6.4 Ammonia and nitrite

Fish excrete most of the nitrogenous wastes through gills in form of ammonia (El-Sayed 2006) which exists in two forms: un-ionized NH_3, that is toxic to fish as well as ionized NH_4^+ which is non- toxic (Chervinski 1982). NH_4^+ is non-toxic to tilapia but prolonged exposure to elevated levels of nitrate may decrease immune response and induced mortality (Plumb 1997). Un-ionised ammonia is vastly toxic to fish as it disturbs the physiological functions of the fish, leading to growth retardation (El-

Sayed 2006). The toxicity of ammonia depends on the concentration of pH, dissolved oxygen and carbon dioxide (El- Sayed 2006) as it increases with decreasing dissolved oxygen and decreases with an increasing carbon dioxide (Chervinski 1982). Massive mortality and reduced growth of tilapia occur when they are exposed to water with un-ionised ammonia in the range of 0.07 and 0.14 $UINH_3$/L (El – Sayed 2006). In support of ammonia toxicity, Ahmed et al. (1992) reported that Nile tilapia exposed to ammonia has lower number of red blood cells and haemolytic anaemia leading to a significant reduction in blood oxygen which enhances ammonia. The effect of ammonia on tilapia performance is also related to water pH, in such a way that, it reduces the specific growth rate of fish when exposed to 0.91 mg / L NH_3 – N at pH 9 (Hargreaves and Kucuk 2001). It is therefore recommended that the NH_3 – N concentration should be maintained below 0.1 UAI – N mg / L (El – Sayed 2006) to prevent mortality as well as enhance growth in fish.

2.6.5 Salinity

The increasing market demand for tilapia and the availability of vast brackish and seawater resources have led to the introduction of these species at large scales (Iqbal et al. 2012). All the commercial valued tilapias (for instance *O. andersonii, O. mossanbicus, and O. niloticus, O. aeureus* and *O. spirulus*) are freshwater species, but can tolerate brackish water (Omeje 2006). Studies have reported that tilapia species have different salinity concentration preferences for in instance; *O. andersonii and O. mossanbicus* can tolerate brackish water up to 20 ppt while *O. niloticus* can only endure salinities up to 15 ppt (Omeje 2006). Moreover, *O. mossanbicus* and *O. andersonii* spawn and grow normally at a water salinity of 49‰ and their fry and fingerling survive and grow gradually at 69‰ (Whitefield and

Blaber 1979). *O. niloticus* and *O. aureus* are least tolerant of saline water but can grow well at the salinity of up to 36‰ - 44‰ while reproduction occurs at 19‰ (El-Sayed 2006). Based on the results reported, *O. andersonii* and *O. mossanbicus* are usually the preferred choice for culture in salt water as they exhibit high tolerance of sanities levels compared to other species (Philipart and Ruwet 1982; Popma and Lovshin 1995).

2.7 Tilapia culture

Tilapia species are widely cultured because they possess all the desired valuable characteristics including high growth rates, ability to spawn in captivity and acceptance of natural and artificial feed, efficient food convention, adaptability to a wide range of environment conditions as well as resistance to diseases under culture conditions (El-Sayed 2006; Phelps and Popma 2000). The first trials of tilapia culture were recorded in Africa in the 1920s (El-Sayed 2006; Shelton and Popma 2006), which later on exploded due to their introduction into tropic, subtropical and temperate regions of the world during the second half of the 20th century (Pillay 1990). The introduction of tilapia into aforementioned areas was for research and recreational purposes, farming as well as to control aquatic weeds. The adaptability of tilapia to a wide range of culture conditions allow this species to be an excellent species to culture as well as exploited as a food fish for the increasing population of Sub-Saharan Africa (Omeje 2016). However, aquaculture production performances were reported to be hindered by the precocious maturity and frequent breeding behaviour of most tilapia species, which in turn results in overpopulation and consequent stunted growth, production of uneven sizes of fish in aquaculture, low

marketable sized fish, reduced survival rate and increased stress in fish making the susceptible to diseases (Varadaraj and Pandian 1990; Popma and Masser 1999; Toguyeni et al. 2002). The concept of monosex culture production of tilapia (preferably male), which subsequently improve yield and economic returns presents an opportunity to overcome the limitation of precocious breeding (Omeje 2016). The establishment of the preferred population (all male tilapia) is based on the fact that male tilapia exhibits rapid growth (Beardmore et al. 2001), spend less energy in reproduction compared to female (Popma and Masser 1999) as well as offer a potential to rheostat undesirable spawning, which will contribute to minimizing stunted growth and thus optimizing the time to harvest (Omeje 2016). Frequent breeding behaviour and precocious maturity of tilapia have enthused the development of various techniques (including hand sorting of sexes, hybridization, temperature, genetic manipulation and hormonal induction) to control spawning in order to achieve all male population with good marketable sized fish (Beardmore et al. 2001; Abad et al 2007; Ampofo 2013).

2.9 Methods to control detrimental spawning in tilapia culture

2.9.1 Temperature

Environmental factors such as temperature and pH were reported to influence sex ratio in teleost species such as *Oreochromis aureus* (Blue Tilapia), particularly during the stage of sexually differentiation (Disperse and Melard 1998; Guerrero-Este'vez and Moreno-Mendoza 2010; Vinas et al. 2013). Sex ratio is influenced by an increase or decrease in water temperature, which consequently modifies the phenotypic sex and shifts the sex ratio to a particular sex (Desprez and Melard 1998). Elevation of culture temperature was described to induce masculinization-based sex ratio while reduced temperature predisposed to feminization (Omeje 2016). In

support, Khater et al. (2017) reported a proportion of 91.50% males achieved when Nile tilapia exposed at high temperature (35°C) during the period of sex differentiation. Azaza et al. (2008) showed a production of 64.2 – 80% masculinization, achieved in tilapia exposed to high temperature of 36.9°C. Similarly, Altena and Horstegen-Schwark (2002) observed a proportion of 79.1% male Nile tilapia treated at 36°C compared to the control reared at 28 °C (54.1%). In contrast, Mair et al. (1990) observed that *O. mossambicus* exposed to a cold temperature (19°C) had a significant excess of males 89% compared to the control reared at 28°C (0%). The author further revealed that in another experiment, a high proportion of *O. aureus* males (97%) reared at the control (29°C) was observed compared to the 80% of females obtained at a warm temperature (32°C). Equally important, the influence of hydrogen ion concentration on sexual differentiation in fish was reported by Guerrero-Este'vez and Moreno-Mendoza (2010), who discovered that high pH (7.9) affect the population being skewed in favour of females while a lower pH (6.2) favours the development of male population.

2.9.2 Hand sexing

Production of all male tilapia has been undertaken manually through separation of the sexes before sexually maturity and before spawning take place. Separation of sexes is carried out by visual inspecting the external genital papilla of each fish' underneath with the aid of dye applied to the papillae of each fish with the total length of 10 cm and weight of about 20 g (El Sayed et al. 2003; Rakocy and McGinty 2005. Dye is essentially used to highlight the papilla structure of the external genitalia of fish in order to improve the visibility since it is not easy to sometimes distinguish the ovarian opening of fish during sexing (Fortes 2005; Fuentes et al. 2013). Male fish can be distinguished from female by means of

assessing the number of openings in the urogenital papillae whereby the genital for the male is simple, pointed and contains a single urogenital papilla whereas the papillae for the female is rounded and contains two openings large enough to allow eggs to be ejected during mating (Rothbard and Pruginin 1975; El-Sayed, 2006). Manual sorting was first to be employed in aquaculture and has been practiced by farmers with few financial resources and little culturing experience (Pandian and Varadaraj 1990). The technique appears to be simple but it is rarely used in nowadays (Penmann and MacAndrew 2000) because it is labour intensive, tedious, stresses fish (Hickling 1963; Beardmore et al. 2001), and produces inaccurate results due to human errors leading to reduced production of required gender (Rakocy and McGinty 2005).

2.9.3 Hybridization

The phenomenon of producing all male progeny through hybridization of two species was discovered in Israel when tilapia breeders revealed that offspring from certain species tends to have high male ratio than females (Pruginin et al. 1975). The technique is used by aquaculturist to produce aquatic organisms with desired traits and has been successfully applied in Japan to produce all male progeny desired to reduce unwanted natural reproduction and control irregular marketable size in the grow out ponds (Marengoni et al.1998). The production of all male tilapia hybrid is achieved by crossing two mouth brooders, which are closely related but distinct subspecies to improve genetics of the fish (Beardmore et al. 2001). Most of the crosses occurred in the tilapias, particularly *Oreochromis* species (for instance male *O. aureus* × female *O. niloticus* and male *O. hornorum* × female *O. mossanbicus)* were reported to be produce 95% - 100% male progenies (Hickling 1960; Wohlfarth

1994; Rosenstein and Hulata 1994). However, other crosses (for instance male *O. niloticus* × female *O. aureus* and male *O. mossanbicus* × female *O. aureus*) have resulted in 50% - 98% male, a drop in the production percentage of all male progeny (Pruginin 1967; Pierce 1980; El – Zaeem and Salam 2013). The failure to produce all male progeny has been attributed to the introduction of hybrids into brood stock pond, inability to control inbreeding, poor management of brood stocks as well as incapability to maintain correct parents, which will produce 100% male offspring (Beardmore et al. 2001). Despite the constraints associated with hybridization, it saves space, time and feeds (Fortes 2005). However, it is still not a perfect solution to the detrimental production of tilapia in aquaculture.

2.9.4 Hormone - induced sex reversal

Hormonal sex reversal is widely used in aquaculture production to convert genotypic female into phenotypic male (Toguyeni et al. 2002; Kefi et al. 2012; Asad et al. 2010), or vice versa, due to the limitation of hand sexing and hybridization. In masculinization, the female fish are induced to develop into phenotypic male and function as male but they possess female genotype (Pandian and Varadaraj 1987). For the production of feminization, the male fish are induced to develop into phenotypic female and function as female but they possess male genotype (Varadaraj and Pandian 1990; Omeje 2016). The gonadal differentiation in *Oreochromis* fry occur between 28 - 48 days' post hatch (Clemens and Inslee 1968; Guerrero 1975; Macintosh et al. 1985; Varadaraj and Pandian 1987; Guerrero and Guerrero 1988; Green et al. 1997) and it is this period the manipulation of the sex ratio can take place. The hormones are usually incorporated into the fish feed and administered to the fry at very larval stages for sufficient time to enable sex reversal. For

masculinization production, 17 α methyl testosterone is frequently used and can be performed through submerging of eggs in different concentration or incorporated into the fish feed and administered to the fish orally for different periods (El-Sayed, 2006). However, the best results have been reported at the dose rate of 30 - 70 mg / kg administered for about 25-50 (Green et al. 1997). In support, Kefi et al. (2012) found out that, the administration of 60 mg 17 α methyltestosterone / kg feed for 30 days produced 94.4% males in *Oreochromis andersonii*. Guerrero (1975) revealed that 98% males of *O. mossanbicus* were obtained at the dose of 30 mg / kg administered for 18 days. Asad et al. (2010) demonstrated that, 100% male tilapia were observed at 40% crude protein diets with 70 α methyltestosterone mg / kg of feed administered for 30 days. Besides its effectiveness, the oral administration was discovered to be laborious and have possible effects on the workers and environment, which resulted to the public criticism. Sex reversal by egg immersion, which produce highest percentage of sex reversed male, substantial decreases the duration of treatment and reduces the possible effects of hormones on the workers (Gale et al. 1999; Beardmore et al. 2001; Cagauan et al. 2004), has attracted attention as a successful method to elucidate the above mention problem. Fitzpatrick et al. (2008) found out that, the immersion of Nile tilapia in 0.5 mg / L for 2 hours each between day 10 and 13 after hatching caused 90% masculinization. The exposure of Nile Tilapia fry to 0.5 mg / L to 17 α methyl testosterones for three hours on two days after hatching resulted in 93% males (Gale, et al. 1996). This alternative technique may be a greater help in aquaculture because it controls sex reversal and employ artificial incubators in hatcheries, but the results are worse compared to hormone on feed and could yield to an uncontrolled reproduction. Despite the outstanding performance of the method, the use of sex hormone treated fish is prohibited in

various countries since the hormone residues remaining in the meat may adversely affect the environment and human health (Curtis et al. 1991; Khalil et al. 2011; El-Greisy and El-Gamal 2012; Singh 2013). Consequently, the use of hormones for sex reversal of tilapia destined for human consumption is either licenced in the USA (Penman and MacAndrew 2000) or banned in India and European Union under directive 96/22/EC article 5, which also prohibits import of animal products produced (Stadtlander et al. 2012).

2.9.5 Plants based phytochemicals as reproduction inhibitors

The exploration for alternative techniques to control reproduction in tilapia culture has led to the consideration of the use of medicinal plants that are safe, biodegraded, environmentally friendly compared to synthetic drugs (Ampofo-Yeboah 2013**)** and have been successfully used to inhibit reproduction in fish and offer a solution to the problem in aquaculture (Lohija and Goyal 1992). Medicinal plants (for instance *Carica papaya, Aloe vera, Azadiracta indica, Tribulus terrestris, Moringa oleifera* and *Hibiscus Rosa Sinensis*) were reported to contain phytochemicals (including Isoflavones, coumestans and lignans (Clotfelter and Rodriguez 2006), which have pharmacological properties and can be used as reproductive inhibitory agents (Jegede 2008; Abdelhak 2013; Ampofo-Yeboah 2013; Gabriel 2015). Phytochemicals are naturally occurring non-steroidal plant compounds that have the ability to control endocrines system by exhibiting estrogenic or androgenic activity (Rearick et al. 2014) because of the structural similarity with estradiol (17- β-estradiol (Younes and Honma 2011). The compounds exert their effects primarily through binding and blocking or inactivating the receptor sites against oestrogen, which may affect the bioavailability of sex hormones by stimulating the synthesis of hormone – binding globulin (Ribeiro et al. 2012). It has been hypothesized that phytochemicals exist within plants as a natural defence against overpopulation of herbivore animals by controlling fertility (Hughes 1988; Nakamura et al. 2007). The hormones secreted by plants exert their effects by modulating the fertility of animals that may eat them to reduce further attack (Hughes 1988; Nakamura et al. 2007).

Some of the phytochemicals were reported to cause sterility in male rats (Kobayashi et al. 2008) and control prolific breeding of *O. niloticus* (Ekanem and Okonkwo 2003). A study by Nkeiruka and Chinaka (2013) showed that, crude extract fed to

male rats at high dosage deteriorated the quantity and quality of the sperms as well resulted in weight loss due to toxicity. The results obtained by Obaroh and Nzeh (2013) revealed that, crude extract of *Azadiracta indica* leaves can control prolific breeding by inhibiting hatching at the dose of 0.5 g / kg feed. Jegede (2010) demonstrated that, *Hibiscus Rosa sinensis* leaves may be effective as a reproduction inhibitor in *O. niloticus* as they destruct the testes and ovary tissue of the fish. Observations by Kavitha and Subramanian (2011) revealed that *Tribulus terrestris* induces the testicular enzyme activity which may aid in the male reproduction functions. A percentage of 80.42% male *Claris gariepinus* was obtained at the administration of 9 g T. *terrestris* per kg feed for 30 days (Cek and Turan 2007). Using similar herbal plant, results of 78.96 % and 87.77% of masculization and survival, respectively, were obtained (Janalizadeh et al. 2018).

2.9.6 Pawpaw as a source of phytochemicals

Carica papaya Linn (Caricaceae), commonly known as Pawpaw or Papaya, refers to short-lived evergreen pachycaul herbaceous plant, which reaches the height of up to 10 m (Verma et al. 2017) and bear hermaphrodic flowers, which have both pistils and stamen or monocious, which bear separate male and female flowers on the same plant (Medina 2010; Vij and Prashar 2015). The family Caricaceae comprises of about 35 species in currently 6 genera of which 32 species (including *Carica papaya*) primarily native in Central and South America and two in Africa (Carvalho and Renner 2014). Papaya was among the first flowering plants selected for full genome sequencing and genomic studies because of its economic importance (Liu et al. 2004; Ming et al. 2008; Yu et al. 2008; Zhang et al. 2008; Wu et al. 2010; VanBuren and Ming 2013; Carvalho and Renner 2014), and it is commonly cultivated throughout especially in Sub-Saharan Africa, tropical and sub-tropical temperate regions (for

instance Egypt) due to its delicious and nutritive fruits (Carvalho and Renner 2014). The popularity of the species is due to its unripe pulp, seeds and leaves which comprise of phytochemicals (for instance saponins, alkaloids, terpenoids, flavonoids, glycosides, steroids and cardenolides) (Lohiya et al. 2000; Oloyede 2005; Ezike et al. 2009; Oloyede 2005; Ezike et al. 2009), calcium, vitamin A, B_1, B_2 & C (De Oliveira and Vitória 2011) as well as the enzymes called papain (Mitchel et al.1970; Jackwheeler 2003) and Chymopapain A and B (Watson et al. 1990), which all promote healthy growth, and aid in proper function of muscles as well as metabolism of protein, carbohydrate and fat (Su et al. 2009; Ekanem and Okonkwo 2013;Yogiraj et al. 2014). Apart from nutritive aspects, the enzyme papain and phytochemicals were reported to contain antifungal, antiviral and antibacterial properties (Jimenez-Arellanes 2003; Abad et al. 2007; Su et al. 2009; Islam et al. 2010; Verma et al. 2017) exhibited effective medicinal properties for example skin adhesion reduction, fertility control, treatment of ulcers, hypertension, diabetes mellitus, hypercholesterolemia and sexually transmitted diseases for example gonorrhoea infections, syphilis and amoebic dysentery infections (Gill 1992; Verma et al. 2006; De Oliveira and Vitória 2011; Wet et al. 2012; Abdelhak 2013). Studies in animals have reported that papaya seeds can control reproduction by triggering temporary infertility and irregular oestrous cycles which is reversible when the treatment is withdrawn (Ekanem and Okorokwo 2003). In general, sterility is elicited by high dosage of papaya seeds which are believed to be toxic as they contain the enzyme papain as well as active ingredients (i.e. carotenoids and oleanolic glycoside), which have been reported to cause sterility in male rats (Kobayashi et al. 2008), control prolific breeding of *O. niloticus* (Ekanem and Okonkwo 2003) as well as break down a membrane vital for the development of the foetus as well as supress the

progesterone, a hormone needed to prepare the uterus for contraception and maintain pregnancy (Aravind et al. 2013).

Additionally, a study by Nkeiruka and Chinaka (2013) showed that, crude extract fed to male rats at high dosage deteriorated the quantity and quality of the sperms as well resulted in weight loss due to toxicity. Histological observations of gonads in *O. niloticus* fed diets containing PSM revealed that, pawpaw seeds may be effective as sterility - inducing agents at high dosage as they disintegrate the testes and ovaries leading to the devoid of spermatids and oocytes, respectively (Jegede and Fagbenro 2008; Ekanem and Okonkwo 2003). Farrag et al. (2013) observed that papaw seeds may improve the growth performance, survival and food conversion rate when the feed is administered to the fry two weeks after hatching. A high percentage of male fish (77.8%) was obtained in the study on the effect of pawpaw seed powder on the sex differentiation, growth and survival of *O. mossanbicus* fry (Omeje 2016). Based on these findings, the use of plant extracts, which are safe, biodegradable and environmentally friendly, could be a possible solution to control undesirable recruitment in *O. andersonii*.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Experimental diet

3.1.1 Pawpaw seed powder and diet

Ripe fruits of pawpaw (*C. papaya*) were procured from Food Lovers Market (Fruit & Veg city Namibia (Pty) Ltd), Swakopmund, Namibia. Fresh seeds were collected from the fruits and rinsed in water to remove the attached membrane of the fruit. The seeds were then spread on newspapers and sun dried. The dried seeds were grounded into powdery form using a mortar and pestle, packed in containers and stored in a cool dry place until later use. A basal diet (32% Crude protein) was prepared with locally available ingredients (Table 1) (Loum 2013). Experimental diets (7 kg per group) were prepared by incorporating *Carica papaya* powder at varying levels (5, 15, 25 and 35 g / kg basal diet) to the control diet (basal diet) and then thoroughly mixed by hand for 5 minutes. To obtain stiff dough and enable pelleting of the diet, a sufficient amount of water was added to the diet during mixing. The pellets were produced using a pelleting machine with 4.5 mm diameter and sun dried for 96 hours.

Figure 1: Fresh fruits and dried seeds of *C. papaya* procured for the preparation of experimental seed powder used in the experiment. Photographs taken by Linda Iipinge.

Table 1: Nutrient composition and proximate analysis (%) of the experimental diets fed to *Oreochromis andersonii* for 120 days.

Ingredient		**MT (g / kg basal diet)**	**Dietary *Carica papaya* (100 g / kg basal diet)**			
	Control (%)	0.06	5	15	25	35
Fishmeal	30.5	30.5	30.5	30.5	30.5	30.5
Peas	14	14	14	14	14	14
Maize meal	32.5	32.5	32.5	32.5	32.5	32.5
Wheat flour	8	8	8	8	8	8
Pearl millet flour	9.5	9.5	9.5	9.5	9.5	9.5
Vegetable oil	4.5	4.5	4.5	4.5	4.5	4.5
Vitamin premix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix[2]	0.5	0.5	0.5	0.5	0.5	0.5
Carica papaya	0	0	5	15	25	35
Methyltestosterone	0	0.06	0	0	0	0
Total	100	100	100	100	100	100
Nutrient composition			**Proximate analysis (%)**			
Crude protein	31.4	31.4	32	30.7	30.2	30.5
Crude lipid	9.05	9.05	8.11	8.27	8.50	8.37
Ash content	5.78	5.78	5.99	6.62	7.58	5.78
Gross energy KJ/g	17.16	17.16	17.16	17.16	17.16	17.16

Note: 1 Vitamin premix mg / kg = $V_{A,}$ 10 000 000 i.u., V_{D3}, 1 000 000 i.u., V_E 40 000, V_{K3} 2 000, V_{B1} 2 500, V_{B2} 7 000, V_{B3} 25 000, V_{B6} 3 000, V_{B7} 50, V_{B9} 800, V_{B12} 20, V_C 50 000, 2 Mineral premix mg / kg: Calcium Pentothenate 10 000, Lecithin 20 000, Carnitine 2 000, Choline 4 000.

3.1.2 17 α methyltestosterone and diet

A stock solution was prepared by dissolving 60 mg of 17 α methyltestosterone (MT) in 600 ml of 95% ethanol (Kefi et al. 2012). The required amount of stock solution of 60 mg was added to a kg of basal diet powder, mixed thoroughly for the hormone to penetrate and allowed to dry at ambient temperature. The feed for the fry was prepared into powdery form through grounding and sieving using mortar and pestle, packed into containers and stored at -20° C (freezer) until use.

3.2 Experimental fish and experimental set-up

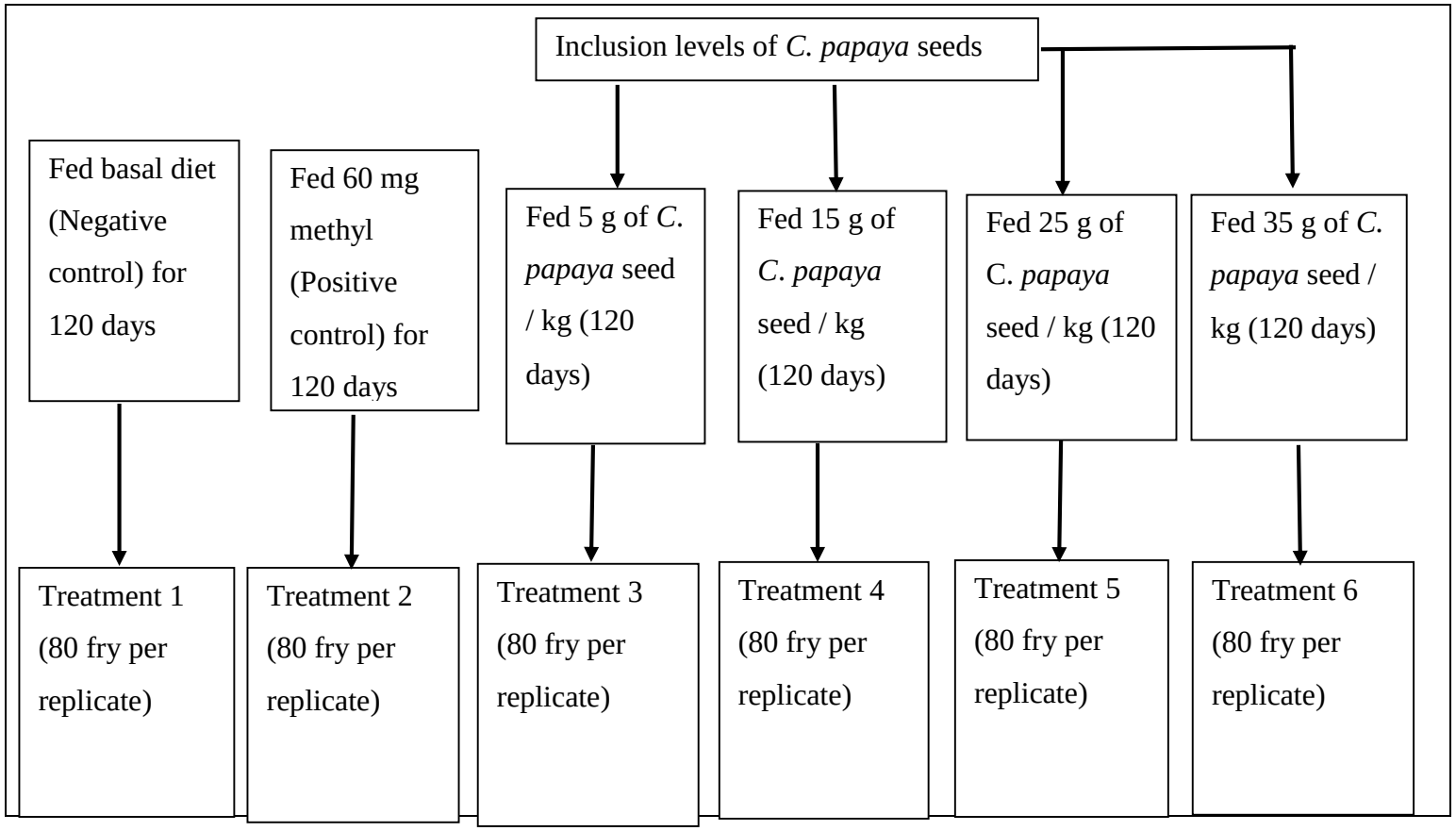
O. andersonii brood stocks, a total number of 26 females with average weight of 176.06 g ± 5.55 and average length of 21.42 cm ± 0.262 and 13 males with average weight of 289.27 g ± 7.84 and average length of 25.60 cm ± 0.257 were kept at Sam Nujoma Marine and Coastal Resources Centre Fresh Water Laboratory and used for spawning. Broodstock were stocked in a cylindrical tank of about 1000 litres and sorted in the ratio of 1:2 (male: female) for spawning. The brood stocks were fed twice in a day with a basal diet (25% CP) obtained from Onavivi Inland Aquaculture Center. Fertilized eggs were removed from the mouth of the female brooders and artificially incubated and hatched in the aquarium (adopted from Omeje 2016). At 4 - 5 days most of the eggs were hatched and had yolk sacs attached to the body, without external feeding. Fry were kept in the aquarium for 9 – 10 days to absorb their yolk sacs, before starting with external feeding. (This was carried out in accordance with research protocols of Omeje 2016).

After yolk sac absorption, the swim–up fry (9 days old) were collected from the aquarium using a fine mesh dip scoop net, weighed and randomly distributed in a

Complete Random Design to six triplicated treatments including a control diet (negative control), 60 mg MT diet (positive control) and *C. papaya* incorporated at 5 g/kg diet, 15 g / kg diet, 25 g / kg diet and 35 g / kg diet. There were 6 experimental treatments (replicated thrice) based on the inclusion level of pawpaw seed powder and 17 α-methyltestosterone (Figure 2). Fry with an average initial body weight of 0.04 g / fry were stocked at a density of 80 fry per replicate and were fed four times per day (9h00, 12h00, 15h00 and 18h00) with powdery experimental diets until apparent saturation, which was reduced to three times (10h00, 15h00 and 18h00) after 30 days. The feeding frequency was reduced due to the fact that when the fish grow, their metabolic rates decreases and their stomach becomes big enough to hold all the feed required for the day (El-Sayed 2006). The feeding was carried out from the beginning when the fry were 9 days until the 4^{th} month (120 days), when the fish reached their sexual maturity. The use of experimental fish was in accordance with scientific research protocols of the University of Namibia (Henties Bay, Namibia) and complied with all relevant national and international welfare laws, guidelines and policies. At the end of 120 days, fish were starved for 24 hours prior to sampling to allow feed evacuation from the gastro-intestine tract.

Figure 2: Inclusion levels of *C. papaya* seed meal in the experimental diets fed to the fish during the experiment.

Experimental design



3.3 Maintenance of culture conditions

The experiment was conducted in 18 black bins (each with 200 L holding capacity) with 300 W submersible heaters stationed at the bottom of each culture tank and aerated using a blower, distributing compressed air through a flexible 3.50 mm tube with an air stone connected at an end. Since water quality parameters affect the growth of the fish, they were maintained at their optimum levels throughout the experiment during tilapia culture in order to ensure optimum growth of fish and prevent their effects on the fish growth. Dissolved oxygen (DO) was maintained above 50% saturation ranging from 3.49 – 4.46 mg / L, water temperature was maintained at 28 ± 2°C and pH was maintained within 7.5 – 8.03 log (mol / L) (Eutech multi-parameter, PCD650, Singapore). Water quality parameters were monitored on a daily basis as they are most significant to sex differentiation and growth in fish (Varadaraj et al. 1994; Baroiller and D'cotta 2001). The recorded water quality parameters, displayed in Table 2, where within the acceptable limits for the tilapia culture (Timmons and Losordo 1994). Tanks were cleaned twice a week to improve the quality of the water and to prevent fry from feeding on other food sources such as algae which might develop inside the tanks.

Table 2: Water quality parameters maintained during the rearing of *Oreochromis andersonii* fed experimental diets for 120 days (Mean ± SE).

Parameter	Mean ± SE	Range
Temperature (°C)	28.93 ± 0.11	28.56- 29.19
Dissolved oxygen (mg/L)	3.09 ± 0.36	1.68 – 2.25
pH (log (mol/L)	8.56 ± 0.20	8.15 – 10.37

3.4 Data collection

3.4.1 Growth performance parameters

The total body weight of the 20 fish was recorded at the beginning of the experimental period as well as at the end of the experimental period (120 days), 24 hours after last feeding, on a sample of 20 fish, randomly selected from each replicate using an electronic weighing scale (Mettler Toledo digital scale, SB8001, Columbus, Ohio, USA). Fish growth was assessed in terms of weight gain (WG), absolute growth rate (AGR), specific growth rate (SGR) and condition factor (CF). The mortality in the different treatments was recorded throughout the experimental period. Feed amount was recorded to account for feed utilization parameters such as food conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER) and feed intake (FI) using the following formulae.

1. $Weight\ gain\ (g) = Final\ weight\ (g) - Initial\ weight(g)$
2. $Absolute\ growth\ rate\ (g/day) = \frac{Final\ weight - Initial\ weight}{Time\ (days)}$

Time is the feeding trial period (days).

3. $Specific\ growth\ rate$ (% per day) $= \frac{In\ Final\ weight - In\ Initial\ weight}{Time\ (days)} \times 100$

Time is the feeding trial period (days).

4. $Feed\ intake\ (g/fish) = \frac{Dry\ feed\ intake}{Number\ of\ fish}$

   Feed intake is the amount of feed consumed by fish throughout the period of experiment.

   Feed intake was measured by dividing total feed intake by the number of fish ($n = 80$). Fry were fed differently because they were provided with different experimental diets. Since the fish were fed until satiation, not all the fish finished their meal especially those from the treated groups (15 g / kg PSM and 35g / kg PSM), so when the surplus feed was detected, feeding was reduced to allow fish to finish their meal.

5. $Feed\ conversion\ ratio\ (g/g) = \frac{feed\ intake\ (g)}{Weight\ gain\ (g)}$

6. $Feed\ efficiency\ ratio\ (g/g) = \frac{Weight\ gain\ (g)}{Feed\ intake\ (g)}$

7. $Protein\ efficiency\ ratio\ (g/g) = \frac{Weight\ gain\ (g)}{Amount\ of\ protein\ fed\ (g)}$

Protein efficiency ratio = is an indicator of the quality of protein content in the feed. The protein fed is the sum of protein content from different ingredients used in the formulation of basal diet. (E.g. the same for all groups = crude protein was 32 % / 100 g / kg basal diet). Thus, protein efficiency ratio was calculated by diving the weight of the fish for each group with the protein fed multiplied by the feed intake for each group.

8. $Survival\ rate\ (\%) = \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \times 100$

3.4.2 Organ somatic parameters

Three fish, randomly selected from each treatment were sacrificed and dissected to remove the gonads and liver. Fish were sedated with clove oil before they were

sacrificed during sampling to enhance the welfare of the fish. The length of each fish was measured using a measuring board and fish bodies, livers and gonads were measured using an electronic weighing scale to compute condition factor, hepatosomatic index and gonadosomatic index and viscerosomatic index as shown below:

9. $Condition\ factor\ (\%) = \frac{Weight\ (g)}{Length^3\ (cm)} \times 100$
10. $Hepatosomatic\ index\ (\%) = \frac{Liver\ weight}{Fish\ weight} \times 100$
11. $Gonadosomatic\ index\ (\%) = \frac{Gonad\ weight}{Fish\ weight} \times 100$
12. $Viscerosomatic\ index\ (\%) = \frac{Gutted\ weight}{Body\ weight} \times 100$

Gutted weight is the weight of the fish after guts, gonads and liver were removed.

3.4.3 Determination of sex ratio

Female and male fish from each replicate were identified by inspection of the genital papilla of the fish. The identification was done at the end of 120 days' trial, when the fish were grown to the size that allowed visual sexing by external secondary sex characteristics. Fish were sexed with the aid of manual sorting and each fish sampled was dissected to confirm the sex. Fish were anaesthetized with clove oil before they were sacrificed during sampling to avoid impairment and enhance the welfare of the fish.

3.5 Statistical analysis

Growth performance, feed utilization and organo-somatic parameters were expressed as mean ± standard error (±). One-way Analysis of Variance (ANOVA) was used to

determine if there was a significant difference among the groups. A post hoc test (Duncan's New Multiple Range Test) was further used to statistically determine the differences between means for the treatments at the significance level of 0.05. Observed number of males and females in each dietary treatment were compared to the expected number under an even sex ratio hypothesis with chi-square test. A significance level of 0.05 was used to indicate if the observed ratio deviated significantly from the expected ratio of 1:1 male: female. Differences in male percentage were analysed using Duncan's New Multiple Range Test in order to separate the groups' means at a significance level of 0.05. All the tests were performed using the statistical package for social sciences (SPSS) computer software (version 21, Chicago, II, USA).

CHAPTER 4

4. RESULTS

4.1 Growth performance and feed utilization

Growth performance and feed utilization parameters of *O. andersonii* fry fed 60 mg α-methyltestosterone and graded levels of PSM for 120 days are illustrated in Table 3. The results indicated no significant differences in growth performance and feed utilization parameters between the controls (basal diet and methyltestosterone) and other treatments up to 15 g PSM / kg diet ($P > 0.05$). A reduction in fish growth and deterioration of fish feed capacity was observed ($P < 0.05$) with increasing the inclusion levels of pawpaw seed meal from 15 g to 35 g / kg diet. Condition factor showed significant differences ($P < 0.05$) among the treatments, however no clear trend was evident. HSI showed no significant differences ($P > 0.05$) between the control and other treatments up to the 15 g / kg diet. However, HSI became significantly poorer with increased inclusion levels from 15 g / kg diet to the 35 g / kg diet. VSI and GSI showed no significant differences among the treatments ($P > 0.05$). Dietary *C. papaya* did not significantly affect survival ($P > 0.05$). All fish survived in all treatments.

Table 3: Morphometric parameters (mean ± SE) of 3 months old *O. andersonii* that received diets containing *C. papaya* seed meal incorporated at different levels over a period of 120 days.

			Dietary *Carica papaya* g / kg diet			
Parameter	**Basal diet**	**60 mg MT**	**P5**	**P15**	**P25**	**P35**
FW (g)	41.86^{bc}±2.72	43.19^{c}±3.33	42.80 ^{c} ±1.26	39.44^{abc}±0.48	35.26^{a}±0.41	36.43^{ab}±0.51
WG (g)	41.82^{bc}±2.72	43.14^{c}±3.33	42.76 ^{c}±1.26	39.40^{abc}±0.48	36.22^{a}±0.41	36.39^{ab}±0.51
SGR (% per day)	5.79^{bc}±0.05	5.82^{c}±0.07	5.81^{c}±0.02	5.74^{abc}±0.01	5.65^{a}±0.08	5.68^{ab}±0.01
AGR (g)	0.35^{abc}±0.02	0.36^{c}±0.03	0.36^{bc}±0.01	0.33^{abc}±0.00	0.29^{a}±0.00	0.31^{ab}±0.00
FI (g)	56.02^{a}±3.63	54.66^{a}±2.23	53.81^{a}±2.68	58.82^{a}±1.44	52.98^{a}±1.69	58.03^{a}±0.37
FCR (g)	1.35^{ab}±0.12	1.29^{ab}±0.16	1.26^{a}±0.10	1.49^{ab}±0.52	1.50^{ab}±0.03	1.60^{c}±0.02
FER (g)	0.75^{a}±0.06	0.80^{a}±0.09	0.80^{a}±0.06	0.67^{a}±0.02	0.67^{a}±0.01	0.63^{a}±0.01
PER (g)	2.35^{a}±0.19	2.49^{a}±0.29	2.50^{a}±0.18	2.10^{a}±0.07	2.08^{a}±0.04	1.96^{a}±0.02
CF (%)	0.91^{a}±0.19	1.04^{b}±0.07	0.89^{a}±0.03	0.94^{ab}±0.02	0.89^{a}±0.02	0.96^{ab}±0.02
VSI (%)	11.44^{a}±1.16	15.72^{a}±3.65	10.70^{a}±0.40	10.32^{a}±0.25	10.21^{a}±1.01	11.60^{a}±1.92
HSI (%)	1.89^{ab}±0.18	2.03^{ab}±0.21	2.48^{c}±0.34	2.18^{ab}±0.16	1.61^{ab}±0.19	1.37^{a}±0.14
GSI (%)	1.04^{a}±0.22	2.03^{a}±0.66	1.44^{a}±0.48	1.38^{a}±0.40	1.72^{a}±0.26	1.27^{a}±0.14
SR (%)	100^{a} ± 0.00	100^{a} ± 0.00	100^{a} ± 0.00	100^{a} ± 0.00	100^{a} ± 0.00	100^{a} ± 0.00

Note: [1]Data are expressed as mean ± standard error (M±SE); [2]the difference between the control and the experimental diets were tested at $P < 0.05$. Values with the same superscripts in the same row are not significant different. Where FW = Final weight, WG = Weight gain, SGR = Specific growth rate per day, FI = Feed intake, FCR = Feed conversion ratio, FER = Feed efficiency ratio, PER = Protein efficiency ratio, CF = Condition factor, VSI = Viscerosomatic index, HIS = Hepatosomatic index, GSI = Gonadosomatic index, SR = Survival rate.

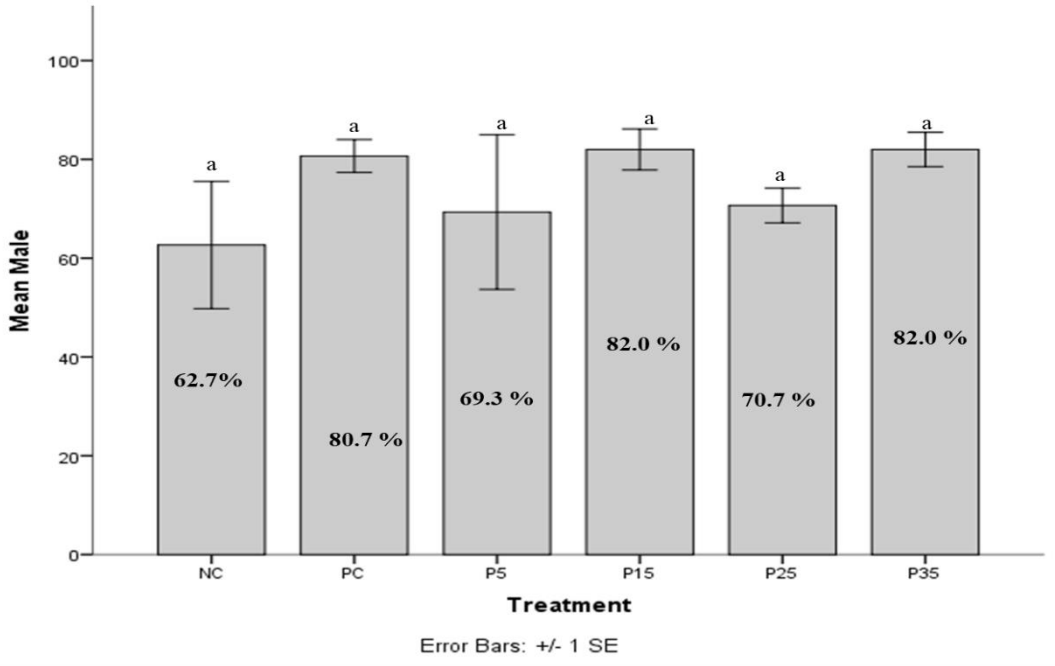
4.2 Effect of pawpaw seed meal on sex ratio

There was a significant influence on sexual differentiation of *O. andersonii* fry after feeding with 60 mg a-methyl testosterone and varying levels of dietary *C. papaya* seed meal for 120 days (Table 4). The results showed a significant deviation (among all the groups) from the expected 1: 1, male: female, sex ratio in favour of males. The control group showed a significant deviation (1.68:1) ($P < 0.05$) from the expected 1: 1, male: female sex ratio. All the treatment categories showed higher percentage of males (but not significantly) compared to control (figure 3). The highest percentage of masculinization (82 ± 4.2) was observed in the fish fed 15 g PSM / kg diet and those fed 35 g of PSM / kg, compared to other treatments.

Table 4: Effect of *C. papaya* seed meal and 17 α-methyltestosterone incorporated as part of a basal diet to masculinize *O. andersonii* fry after 120 days treatment period.

Treatment	Parameter					
	Male %	**Female %**	**N**	**Sex ratio**	**Calculate X^2**	**P value**
BD	63	37	150	1.68:1	9.63	0.002
60mg MT	81	19	150	4.71:1	56.43	< 0.0001
P5	69	31	150	2.26:1	22.43	< 0.0001
P15	82	18	150	4.56:1	61.44	< 0.0001
P25	71	29	150	2.41:1	25.63	< 0.0001
P35	82	18	150	4.56:1	61.44	< 0.0001

Figure 3: The sex ratio (%) of *Oreochromis andersonii* fed diet containing 60 mg of 17 α methyltestosterone and varying inclusion levels of pawpaw seed meal for 120 days.



Note: [1]Data are expressed as mean ± standard error (M±SE); [2]the difference between the control and the experimental diets were tested at $P < 0.05$. Bars with the same superscripts are not significant different. Where NC = Negative control, PC = Positive control, P 5 = 5 g of pawpaw seed meal, p 15 = 15 g of pawpaw seed meal, p 25 = 25 g of pawpaw seed meal, p 35 = 35 g of pawpaw seed meal.

CHAPTER 5

5. DISCUSSION

5.1 Influence of methyl testosterone and pawpaw seed meal on morphological characteristics

In this study, the results showed no growth improvement in the fish fed dietary inclusion levels of *C. papaya* compared to the fish fed basal diet and those fed methyltestosterone. The results indicated deterioration of feed utilization capacity parameters of three spotted tilapia during the treatment period with increasing the dietary inclusion levels from 15 g to 35 g of PSM / kg diet, this consequently reduced the fish growth and organo-somatic indices. These results agree with the findings of Ekanem and Okoronkwo (2003); Abbas and Abbas (2011) who reported that addition of pawpaw seed powder decreased the growth performance of *O. niloticus* during 30 days treatment period. Ampofo-Yeboah (2013) and Omeje (2016) also reported a decrease in growth performance of *O. mossambicus* with increasing the inclusion levels of pawpaw seed powder during 60 days and 120 days treatment period, respectively. In other study, Ugonna et al. (2018) reported that *O. niloticus* fed with basal diet showed the best growth performance, whereas the fish fed with the treated feed showed a significant reduction in growth with increasing the inclusion levels of PSM during 28 days treatment period. The present results are contrary to report by Farrag et al. (2013) who observed a positive effect of pawpaw seed powder on growth performance parameters and better feed utilization in Nile tilapia fry with increasing the pawpaw inclusion levels during 45 days treatment period. This is also contrary to the discoveries of Kefi et al. (2013) who reported an improved in growth performance of *O. andersonii* fed 60 mg of 17 *a* methyltestosterone for 28 days.

Feed conversion ratio is a valuable and powerful tool in aquaculture that allows fish farmers or nutritionist to make wise choices in selecting or estimating the amount feed that will be required in the growing cycles (Anderson and Silva 2003) and aids them in using of feed efficiently to maximize profitability. A low feed conversion ratio is a good indication of high-quality feed and it means that fish utilized the feed better. FAO (2015) reported that the optimum feed conversion ratio level for tilapia species is 1.5. DeLong et al. (2009) stated that the feed conversion ratio for Nile tilapia generally ranges from 1.4 – 1.8: 1. Other studies reported the feed conversion ratio for farmed fish and shrimp is ranged from 1.0 to 2.4 (Tacon 2008; Zuidhof et al. 2014), which have to be lower than that for larger terrestrial animals because they spend less energy on movement, stay upright and regulate their body temperatures due to buoyancy and because most of them are ectothermic (Naylor et al. 2009; Torrissen et al. 2011). In the present study, feed conversion ratio values ranged from 1.26 ± 0.10 to 1.60 ± 0.02, which were within range of feed conversion ratio values reported by (DeLong et al. 2009; Tacon 2008; Zuidhof et al. 2014). A report by Farrag et al. (2013) showed a significant increase ($P > 0.01$) in feed conversion ratio of Nile tilapia by increasing the levels of pawpaw seed meal until treatment 3 (6 g PSM / kg diet) and then significantly decreased ($P < 0.01$) by higher levels of PSM (8 g PSM / kg diet). The present results showed that feed conversion ratio was not significantly influenced ($P > 0.05$) by the pawpaw seed meal for all treatments, except for the fry fed 35 g of *C. papaya* which showed significantly lower feed utilization efficiency than other treatments. This may be due to the presence of carpasemine in the pawpaw seeds, which lowed the palatability of the feed, consequently reduced competition for food and caused deterioration of feed utilization efficiency.

Feeding of fish depends on their sensory capacities to find food, the ability to capture feed, handle and ingest food and physiology which determines how fish digest and process feed (Kestemont and Baras 2001). Feed intake depends on the handling of fish, cleaning of tanks and palatability of the feed. A report by Olaniyi and Salau (2013) discovered that the feed intake for African cat fish fed pawpaw leaf significantly decrease with increasing the inclusion levels of pawpaw. In other study, Farrag et al. (2013) observed a significant increase ($P < 0.01$) in feed intake of Nile tilapia by increasing the levels pawpaw seed meal until treatment 3 (6 g PSM / kg diet) and then significantly decreased ($P > 0.01$) by higher levels of PSM (8 g PSM / kg diet). In the present study, feed intake decreased with increasing the inclusion the pawpaw seed meal for all the treatments. This agrees with Pheng Bunta et al. (2008) who reported that feed intake reduced with reduction in palatability.

Protein efficiency ratio indicates the quality of protein content in the feed. It determines the ability of the protein in the feed to support the growth of the fish and usually higher protein efficiency ratio values are required for better feed utilization of fish. In the present study, protein efficiency ratio values ranged from 1.96 g ± 0.02 to 2.50 g ± 0.18, which were higher than those reported by Ugonna et al. (2018); Puycha et al. (2017) for Nile tilapia fed PSM and Bocourtis catfish fed moringa leaf, respectively. The differences between protein efficiency ratio reported by different studies could be due to the quality of dietary protein used.

Ugonna et al. (2018) hypothesized that reduced growth performance observed in the study could be due to a reduction in palatability of the diets because of increased PSM inclusion. Uneaten feed observed more frequently in PSM treated groups than control due to lowered palatability was reported to be caused by the presence of carpasemine (growth inhibitor), saponins, tannins and phenolic (for instance

flavonoids) in PSM (Lohiya et al. 2000; Pathak et al. 2000; Lohiya et al.2002; Kobayashi et al. 2008; Saxena et al. 2013). These compounds can be toxic to fish especially at high dose (Bureau et al. 1998; Makkar and Becker 1998; Sakai 1999; Makkar et al. 2007; Akinpelu et al. 2012; Vinay et al. 2014; Gabriel 2015). These bioactive compounds may present a bitter taste that might act as feed deterrents (Sakai 1999; Dongmeza et al. 2006) and this may consequently affect growth. The mode of action for instance for saponins include depression of feed intake and reduction in weight gain which has been reported in ruminant animals (Cheeke 1996). Saponins are also believed to hinder the intestinal uptake of nutrients (Johnson et al. 1986) such as minerals (Southon et al. 1988), vitamins (Jenkins and Atwal 1994) and dietary fat by inhibiting pancreatic lipase activity (Han et al. 2001) as well as reduce the palatability and lower the digestibility leading to poor utilization of protein and amino acid (Shimoyamada et al. 1998).

Tannins and other phenolic substances were also reported to have adverse effects in fish as they reduce feed intake, growth and nutrient availability as well as increase the endogenous losses of nitrogen through faeces (Makkar and Becker 1998). The mechanism of action of tannins and phenolic substances includes creating complexes with proteins, thereby inhibiting their uptake as well as enzymes, thus preventing protein digestion (Al-Owafeir 1999; Richter et al 2003; Puycha et al. 2017). This hypothesis was supported by the findings of Al-Owafeir (1999) which reported a significant growth reduction in African catfish fed with the diets containing 0.27% level of tannic acid. A study by Becker and Makkar (1999) has also reported a reduced feed acceptability by common carp after 4 weeks of feeding with the inclusion level of 2% of hydrolysable tannin in the diet. Other factors as reported by

Omeje (2016) could be that the fish had not been cultured for a long period in a conducive environment like ponds which will allow enhanced growth.

Cone (1989) indicated that the relationship between fish weight and length (condition factors) frequently used compared the effect of biotic and abiotic on the health or well-being of the fish. Condition factors used in study compared the effect of active ingredients in the pawpaw seeds on the physiological state of the *O. andersonii*. The results showed no evident trend, signifying that the influence of pawpaw seed meal in the diet on the health of the fish is not that clear. GSI represents the relationship between the gonad weight and body weight (Horstegen-Schwark and Langholz 1998) and it is an important tool in science as it is used to indicate the gonadal maturation in fish (Omeje 2016). The results obtained indicated that gonadosomatic index was not significantly influenced by the pawpaw inclusion levels. Tyler and Sumpter (1996) reported that it is infrequently for the gonadosomatic index of *Oreochromis* species to exceed 10% of body weight, however it fluctuates between 2 and 10% between spawning seasons. Similarly, Peters (1983) reported that mouth- brooding *Oreochromis* species such as *O. andersonii* and *O. mossambicus* exhibit gonadosomatic index between 4.6% and 10.2%. The gonadosomatic index observed in the study for the fish treated with inclusion levels of pawpaw did not fluctuate between 2 and 10%, implying that the fish were not ready for spawning. Kobayashi et al. (2008) reported that extracts from various parts of the pawpaw tree decreased the testicular of Wistar rats when administered orally for eight weeks. The GSI for the fish treated with methyl testosterone fluctuated between 2 and 10%, in agreement with Tyler and Sumpter (1996). Phelps and Popma (2000) reported that under favourable conditions most tilapia fish for instance *O. andersonii* and *O. mossanbicus* reaches maturity within 3 months at the size less than 100 g. In the

present study, spawning was observed in the fish fed basal diet after 3 months, though their GSI did not fluctuate between 2 and 10. This observation was reported to be due to the fact that the fish used in the experiment were sexually matured (35 g – 43 g), thus had the capacity to reproduce.

Liver is a useful organ which is used as a target for the metabolism in the fish body, thus hepatosomatic index is a useful biomarker to detect the hazardous effects of the environmental stressors (Pait and Nelson 2003). The inspection of liver is relevant as it plays a significant role in detoxifying of various metabolites as well as excretion of xenobiotic compounds (Rocha and Monteiro 1999). In the study, the mean liver weigh of *O. andersonii* decreased with additional inclusion levels of pawpaw. Ekanem and Okoronkwo (2003) observed discoloration of the liver in *O. niloticus* fed higher dietary pawpaw seed meal levels for 60 days. The reduction of hepatosomatic index observed could be subjected to the concentration of phenol present in the fish diet which is exhibit degenerative effects at high dosage. Sadekarpawar and Parikh (2013) states that the liver happens to be the most affected tissue as it tries to detoxify the toxicant that enters the liver through blood circulation, where it gets transformed and excreted through bile excretion secretion or get transferred to the kidney for filtration and transformation. Kime (1998) suggested that when the exposure happens to be for a long term, liver morphology and enzymatic activity will be altered. The hypothesis was supported by the findings of Barse et al. (2006); Mir et al. (2012) who reported a degenerative effect on Carp (*Cyprinus carpio*) subjected to 4-tert-butylphenol.

Survival rate is the percentage of fish in the treatment period still alive for a period of time (120 days) after exposed to the diet treated with methyl testosterone and pawpaw seed meal. In the study, survival rate of the fish was not affected to the

application of the treatments which agrees with the discoveries of Ampofo-Yeboah (2013); Omeje (2016); Ugonna et al. (2018) who reported lower mortality rate in Mozambique tilapia and Nile tilapia, respectively. Omeje (2016) proposed that non-lethal levels of phytochemicals in pawpaw seed meal were the reason for lower mortality observed in the fish. The present study is contrary to the report by Ayotunde and Ofem (2008) who reported highest mortality rate in fish treated with PSM at level of 9.8 g / kg diet. In general, *C. papaya* inhibited fish growth with increasing the inclusion level, implying that it had toxic effect on the fish at high dosage. Therefore, the study fails to reject the null hypothesis, which stated that feeding sexually undifferentiated *O. andersonii* fry with dietary *C. papaya* seed meal for 120 days does not enhance their feed utilization capacity and ultimately improve their growth performance.

5.2 Influence of methyltestosterone and pawpaw seed meal on sex ratio

This study showed that pawpaw seed meal was able to skew the sex ratio in favour of males, from the expected ratio of 1:1 male: female for all the treatments. An increase in male percentages with an increase in dietary *C. papaya* inclusion levels, with the highest degree of masculinization (82%) observed in fry fed 15 g *C. papaya*/kg diet and 35 g *C. papaya*/ kg diet. The results are consistence with the reports of Omeje (2016); Ugonna et al. (2018) who reported an increase in the male percentage with increasing the inclusion levels in *O. mossambicus* and *O. niloticus*, respectively. This is the highest level of masculinization obtained in tilapia fry following *C. papaya* administration, compared to 77.8% masculinization obtained by Omeje (2016) and 65% masculinization reported by Ampofo-Yeboah (2013) in *O. mossambicus*, respectively. From the current study, masculinization effects of dietary *C. papaya* are

even somewhat higher than that of the MT treatment (81%), which is widely used and believed to be an effective method of controlling reproduction in tilapia culture. The fact that PSM inclusion levels resulted in an increase in percentage of male tilapia indicates that PSM is promising for use in commercial production. The inclusion level of 35 g PSM / kg diet (with the highest percentage of male) resulted in 18% female fish which still present an opportunity to produce precociously which will result in the overcrowding of pond systems to occur. In addition, the hormone 17 α methyltestosterone (at the inclusion level of 60 mg MT / kg basal diet) which was used in the present study to skew the sex ratio in favour of males resulted in 81% males and 19% female, M: F ratio of 4.71:1.

It was hypothesised by Pandian and Varadaraj (1987) that the physiological mechanism which prevented a complete or 100% masculinization in this study can be potentially ascribed to feed competition, which resulted in some individuals having less access to the treatments diet due to a dominance hierarchy experienced in the culture system, henceforth justifying the presence of female progeny observed in the treated groups of the present study. However, it is reasonable to believe that reduced palatability as a result of PSM inclusion, which was earlier linked to reduced growth during treatment periods, could have reduced competition for the feed (Solomon and Okomoda, 2018). Another reason for incomplete 100% male may be due to paradoxical feminization. Alonso et al. (2001) reported that anabolic steroid such as methyl testosterone which is a synthetic androgen, can be converted by means of cytochrome P450 enzyme to form estrogen, which is a feminizing hormone. The aromatization of especially an excess of androgen to estrogen can paradoxically cause the feminization instead of the desirable masculinization in some fish (Omeje 2016).

In addition, a failure to produce 100% masculinization can be also influenced by water quality parameters for instance temperature, pH and dissolved oxygen of the culture systems (Varadaraj et al. 1994). Among the abovementioned factors, temperature is the one considered to be most effective in sex reversal compared to other factors (Baroiller and D'cotta 2001) as it decreases the aromatase activity, thereby predisposing to masculinization (D'cotta et al. 2001). The water quality parameters recorded during the experiment illustrated that water temperature, pH and dissolve oxygen varied between 28.56 – 29.19 °C (mean ± SD: 28.93 ± 0.11), 8.15 – 10.37 (mean ± SD: 8.56 ± 0.02) and 1.68 – 2.25 mg / L (mean ± SD: 3.09 ± 0.36 mg / L), respectively. The water quality parameters were within the tolerable limit for fish culture (Timmons and Losordo 1994). The water temperature observed in the study was potentially suboptimal to effectively allow sex reversal to take place. The hypothesis was supported also by the results of Khater et al. (2017) who reported a proportion of 91.50% males achieved when Nile tilapia exposed at high temperature (35°C) during the period of sex differentiation. Similarly, Altena and Horstegen-Schwark (2002) observed a proportion of 79.1% male Nile tilapia treated at 36°C compared to the control reared at 28 °C (54.1%). In other study, Azaza et al. (2008) showed a production of 64.2 – 80% masculinization, achieved in tilapia exposed to high temperature of 36.9°C.

A significant increase in the observed percentage of males is an indication that pawpaw seed meal used in this experiment contain some phytochemicals (β-sitosterol, saponins and flavonoids) (Krishna et al. 2008), which had their effect on gonad sex differentiation in juvenile tilapia. One of the active ingredients present in pawpaw seeds is oleanolic glycoside (Kobayashi et al. 2008), which was reported to cause sterility in albino rats (Udoh and Kekinde 1999) and hence could have caused

masculinization in treated fish in this study. Omeje (2016) reported that phytochemicals which are obtained through dietary administration of PSM cause changes in sex ratio once they interact with the endogenous hormones. Ampofo-Yeboah (2013) hypothesised that the mode of action through which phytochemicals in the pawpaw seeds could act on the cellular level to cause sexual differentiation process has not been established, however two possible scenarios have been adapted for expression of phytoestrogen in endocrine modulation. The information on the two scenarios has been reported in study by Sirotkin and Harrath (2014), which has reviewed the physiological and health effects of phytochemicals and mechanism of their action.

One of the scenarios is through competition for the estrogenic nuclear receptors (α and β ER) that could possibly mimic the sex reversal effects of androgen treatments in fish (Dabrowsi et al. 2005; Rodriguez Montes de Oca 2005; Moutsatsou 2007). Notable attempts carried out on chemical structure of plants indicated that some phytochemicals have structures similar to that of natural steroid hormones, therefore it can mimic, compete and displace endogenous estrogens from binding sites on estrogen receptor sites (Moutsautsou 2007). They then can act as antiestrogens or weak estrogenic thereby eliciting their biological activity (Omeje 2016). Mathew et al. (2000) reported that activity of estrogens in target cells manifest itself through binding to estrogen receptors. Since the phytochemicals for instance quercetin, genistein and diadzein exhibit structure similar to androgens and estrogens they have a capacity to mimic, compete and displace endogenous estrogens from binding sites on estrogenic receptors (Moutsautsou 2007; Pilsakova et al. 2010). A second scenario is through blocking the biosynthesis and action of estrogenic by inhibition of aromatase activity and other steroid metabolism related enzymes (Adlercreutz et

al. 1987; Berrino et al. 2001; Pilsakova et al. 2010; Ribeiro et al. 2012). According to Omeje (2016) sex reversal is possible because ovarian differentiation commences at an earlier stage compared to testicular tissues. Sexual differentiation into ovarian cells occurs with biosynthesis of estradiol-17 β from cholesterol through enzymatic activity of aromatase (Nagahama 1994; Nakamura et al. 1998). Thus, estrogenic hormones are products of aromatase and any chemical compounds such as the phytochemicals (β-sitosterol, saponins, oleanolic acid-3-glucoside, genistein, quiercetin and kaempferol) that can block activity of aromatase, supressing estrogenic biosynthesis in cells could switch ovarian development to testicular development (Pelissero et al. 1996; Dabrowski et al. 2004). The hypotheses have been supported by Dabrowski et al. (2005); de Oca (2005); El-Sayeed et al. (2012) who reported that phytochemicals could exert influence on gonadal differentiation of fish. Similarly, Ganzera et al. (2001) reported that saponin (which is also part of the phytochemical) elevated testosterone hormone production in animals. A study by Kobayashi et al. (2008) indicated that pawpaw contains oleanolic glycoside which is part of the above-mentioned phytochemicals, considering the fact that pawpaw can be postulated to be a potential aromatizing agent.

In general, this study demonstrated the possibility of using PSM which is cheap (N$ 20 per pawpaw fruit) to attain sex reversal in three spotted tilapia, an indication that it could be used as an alternative to synthetic hormones used in fish food production, which have been speculated to have possible health issues and expensive (R 1000 – R 2000 per 0.01 g capsule). This information could be of particular useful to fish farmers or aquaculture development especially in rural areas of Sub- Sahara African countries for example Namibia, where the pawpaw is available throughout the year compared to the synthetic hormones that require purchasing from South Africa,

thereby lessening the influence of anabolic steroids on fish consumers, fish culturists and on the environment. Therefore, the study rejects the hypothesis which stated that feeding sexually undifferentiated *O. andersonii* fry with different dosage of dietary *C. papaya* seed powder for 120 days does not affect their sexual differentiation by altering their sex ratio toward male populations.

CHAPTER 6: CONCLUSION AND RECOMMEDATIONS

6.1 CONCLUSION

The present study demonstrated that dietary *C. papaya* seed extracts significantly decreased the growth and deteriorated feed utilization capacity of sexually undifferentiated *O. andersonii* fry with increasing inclusion dosage from 15 g to 35 g / kg diet. Significant influence of *C. papaya* seed extracts on fish sex ratio was noticed, with the highest degree of masculinization observed in the fish fed the inclusion level of 15 g *C. papaya* / kg diet and 35 g *C. papaya* / kg diet. The present study showed that pawpaw seed meal could be used as a sex reversal agent rather than a growth-promoting agent in three spotted tilapia. In general, it could be concluded that using pawpaw seeds which are cheaper, biodegradable and easy to obtain could be incorporated into fish feed with adjusted amount and be used by farmers to control breeding of tilapia fish instead of unfavourable synthetic sex reversal hormones use (which many scientists have raised concerns over possible bioaccumulation) however, it should be supplied with know and accurate amount to avoid adverse effects on fish which could occurred due to the strong active ingredients of pawpaw.

6.2 RECOMMENDATIONS

It is suggested that future studies should be conducted to determine the physiological response of *O. andersonii* to varying inclusion levels of pawpaw seed meal in the diet during the recovery periods. Since poor growth was observed with increasing the inclusion levels, the study recommends further studies to conduct experiments around 5 g but not ≥ 15 g of *C. papaya* with large sample size to determine the effect of pawpaw seed meal in fish growth and sex reversal. It may be also interesting to

determine the effectiveness of *C. papaya* seed meal on gonad histology in relation to sex reversal and growth performance of fish as well as to focus on the bioaccumulation of *C. papaya* seed meal contents on the liver, blood haematological and biochemical profile in order to determine the effects of phytochemicals on their architectural integrity. Future studies need to isolate the pawpaw active ingredients and determine which one is responsible for promoting growth and sex reversal in tilapia. In addition, further studies on the interaction of the treatments with environmental factors (for instance water temperature) as well as to optimize dosage levels of *C. papaya* seeds as a dietary supplement in aquaculture need to be established.

CHAPTER 7

7 REFERENCES

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CHAPTER 8

8 APPENDIXES

Appendix 1: List of ingredients used to formulate 10kg of experimental diet fed to *Oreochromis andersonii* for 120 days.

Ingredient	Proportion	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Fishmeal	30.5	3050	3050	3050	3050	3050	3050
Peas	14	1400	1400	1400	1400	1400	1400
Maize meal	32.5	3250	3250	3250	3250	3250	3250
Wheat flour	8	800	800	800	800	800	800
Pearl millet flour	9.5	950	950	950	950	950	950
Vegetable oil	4.5	450	450	450	450	450	450
Vitamin + Mineral premixes	0	100	100	100	100	100	100
Carica papaya	0	0	0	50	150	250	350
Methyl testosterone	0	420	0	0	0	0	0
Total	100	100	100	100	100	100	100

Appendix 2: Raw data on growth performance and feed utilization parameters of *Oreochromis andersonii* fed basal diet, MT and different inclusion levels of *Carica papaya* for a period of 3 months.

Groups	W_f	W_i	WG	SGR	AGR	TF	FI	FCR	FER	PER
Basal diet	38.57	0.04	38.53	5.73	0.32	2477.80	60.97	1.58	0.63	1.20
Basal diet	39.75	0.04	39.71	5.75	0.33	1516.40	48.96	1.23	0.81	1.24
Basal diet	47.25	0.04	47.21	5.90	0.39	2250.00	58.13	1.23	0.81	1.48
MT	36.81	0.04	36.77	5.69	0.31	2290.30	58.63	1.59	0.63	1.15
MT	44.70	0.04	44.66	5.85	0.37	1956.50	54.45	1.22	0.82	1.40
MT	48.05	0.04	48.01	5.91	0.40	1673.00	50.91	1.06	0.94	1.50
5g of PSM	40.95	0.04	40.92	5.78	0.34	2330	59.13	1.45	0.69	1.28

5g of PSM	45.22	0.04	45.18	5.86	0.38	1650.8	50.64	1.12	0.89	1.41
5g of PSM	42.23	0.04	42.19	5.80	0.35	1733	51.66	1.22	0.82	1.32
15g of PSM	40.38	0.04	40.34	5.76	0.34	2094.5	56.18	1.39	0.72	1.26
15g of PSM	39.13	0.04	39.09	5.74	0.33	2490.5	61.13	1.56	0.64	1.22
15g of PSM	38.81	0.04	38.77	5.73	0.32	2331.7	59.15	1.53	0.66	1.21
25g of PSM	35.13	0.04	35.09	5.65	0.29	1726.9	51.59	1.47	0.68	1.10
25g of PSM	36.03	0.04	35.99	5.67	0.30	2107.4	56.34	1.57	0.64	1.12
25g of PSM	34.61	0.04	34.57	5.64	0.30	1681.8	51.02	1.48	0.68	1.08
35g of PSM	37.15	0.04	37.11	5.69	0.31	2301.4	58.77	1.58	0.63	1.16
35g of PSM	36.70	0.04	36.66	5.68	0.31	2207.7	57.60	1.57	0.64	1.15
35g of PSM	35.45	0.04	35.41	5.66	0.30	2218.1	57.73	1.63	0.61	1.11

Note: W_f = Final weight, W_i = Initial weight, WG = Weight gain, SGR = Specific growth rate, AGR = Absolute growth rate, TF = Total feed, FI = Feed intake, FCR =Feed conversion ratio, FER = Feed efficiency ratio, PER = Protein efficiency ratio.

Appendix 3: Raw data on organo-somatic indices and whole-body composition parameters of *Oreochromis andersonii* reared for 120 days.

Groups	**Gonadosomatic index**	**Hepatosomatic index**	**Viscerosomatic index**
Basal diet	0.85	1.42	9.14
Basal diet	1.66	2.40	12.86
Basal diet	0.61	1.84	12.31
MT	3.49	2.14	12.63
MT	0.89	2.36	11.54
MT	1.70	1.60	22.99
5g of PSM	0.88	1.36	10.79
5g of PSM	2.49	3.58	10.68

5g of PSM	0.96	2.51	10.62
15g of PSM	1.85	2.11	9.09
15g of PSM	0.86	2.09	11.34
15g of PSM	1.44	2.35	10.53
25g of PSM	1.96	1.59	11.16
25g of PSM	1.59	1.98	8.73
25g of PSM	1.61	1.25	10.74
35g of PSM	1.21	1.23	9.09
35g of PSM	1.06	1.34	10.34
35g of PSM	1.53	1.53	15.38

Appendix 4: Raw data on the Energy of the ingredients used in the formulation of the experimental diet.

Ingredients	**Energy KJ/100g**	**Energy KJ/g**	**Gross Energy KJ/g**
Fish meal	1979.86	19.7986	6.04
Peas	1820.04	18.2004	2.55
Wheat flour	1464	14.64	1.17
Maize meal	1368	13.68	4.45
Pearl millet flour	1509	15.09	1.43
Vegetable oil	3389	33.89	1.53
Vitamin + Minerals premixes	0	0	0
Total	11529.9	115.30	17.16

Appendix 5: Raw data on the sex ratio of *Oreochromis andersonii* fed with basal diet and different inclusion levels of *Carica papaya* for 3 months.

Groups	**Gender**		**Total**
	Male	**Female**	
Basal diet	44	6	50
Basal diet	27	23	50
Basal diet	23	27	50
MT	42	8	50
MT	37	13	50
MT	42	8	50
5g of pawpaw seed meal	49	1	50
5g of pawpaw seed meal	22	28	50
5g of pawpaw seed meal	33	17	50
15g of pawpaw seed meal	45	5	50
15g of pawpaw seed meal	38	12	50
15g of pawpaw seed meal	40	10	50
25g of pawpaw seed meal	36	14	50
25g of pawpaw seed meal	32	18	50
25g of pawpaw seed meal	38	12	50
35g of pawpaw seed meal	41	9	50
35g of pawpaw seed meal	38	12	50
35g of pawpaw seed meal	44	6	50

Appendix 1: Research permission letter for the study.

CENTRE FOR POSTGRADUATE STUDIES

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UNAM
UNIVERSITY OF NAMIBIA

RESEARCH PERMISSION LETTER

25 October 2017

Student Name: Ms Linda Nuushona Iipinge

Student number: 201036622

Programme: *MSc (Fisheries & Aquatic Sciences)*

Approved research title: Effect of dietary *Carica papaya* crude extracts on gonad morphology, sex ratio and growth performance in *Oreochromis andersonii* fry

TO WHOM IT MAY CONCERN

I hereby confirm that the above mentioned student is registered at the University of Namibia for the programme indicated. The proposed study met all the requirements as stipulated in the University guidelines and has been approved by the relevant committees.

The proposal adheres to ethical principles as per attached Ethical Clearance Certificate. Permission is hereby granted to carry out the research as described in the approved proposal.

Best Regards

Dr M Hedimbi
Director: Centre for Postgraduate Studies
Tel: +264 61 2063275
E-mail: directorpgs@unam.na

25 Oct 17
Date

ANOVA results for morphological parameters						
		Sum of Squares	df	Mean Square	F	Sig.
WG	Between Groups	170.460	5	34.092	3.287	.042
	Within Groups	124.460	12	10.372		
	Total	294.920	17			
SGR	Between Groups	.075	5	.015	3.765	.028
	Within Groups	.048	12	.004		
	Total	.123	17			
AGR	Between Groups	.010	5	.002	3.047	.053
	Within Groups	.008	12	.001		
	Total	.019	17			
FI	Between Groups	81.916	5	16.383	1.081	.419
	Within Groups	181.940	12	15.162		
	Total	263.856	17			
FCR	Between Groups	.269	5	.054	2.073	.140
	Within Groups	.311	12	.026		
	Total	.580	17			
FER	Between Groups	.081	5	.016	2.016	.148
	Within Groups	.096	12	.008		
	Total	.177	17			
PER	Between Groups	.166	5	.033	3.240	.044
	Within Groups	.123	12	.010		
	Total	.290	17			
CF	Between Groups	.050	5	.010	2.028	.146
	Within Groups	.060	12	.005		
	Total	.110	17			
VSI	Between Groups	64.088	5	12.818	1.325	.318

	Within Groups	116.099	12	9.675		
	Total	180.187	17			
HSI	Between Groups	2.414	5	.483	1.605	.232
	Within Groups	3.609	12	.301		
	Total	6.024	17			
GSI	Between Groups	1.819	5	.364	.673	.652
	Within Groups	6.489	12	.541		
	Total	8.307	17			
FW	Between Groups	170.401	5	34.080	3.285	.042
	Within Groups	124.497	12	10.375		
	Total	294.898	17			

FI

Duncan

Groups	N	Subset for alpha = 0.05
		1
p25	3	52.9833
p5	3	53.8100
positive control	3	54.6633
negative control	3	56.0200
p35	3	58.0333
p15	3	58.8200
Sig.		.123

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

WG

Duncan

Groups	N	Subset for alpha = 0.05		
		1	2	3
p25	3	35.2167		
p35	3	36.3933	36.3933	
p15	3	39.4000	39.4000	39.4000
negative control	3		41.8167	41.8167
p5	3			42.7633
positive control	3			43.1467
Sig.		.155	.073	.211

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

SGR

Duncan

Groups	N	Subset for alpha = 0.05		
		1	2	3
p25	3	5.6533		
p35	3	5.6767	5.6767	

p15	3	5.7433	5.7433	5.7433
negative control	3		5.7933	5.7933
p5	3			5.8133
positive control	3			5.8167
Sig.		.123	.052	.212
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 3.000.				

AGR				
Duncan				
Groups	N	Subset for alpha = 0.05		
		1	2	3
p25	3	.2967		
p35	3	.3067	.3067	
p15	3	.3300	.3300	.3300
negative control	3	.3467	.3467	.3467
p5	3		.3567	.3567
positive control	3			.3600
Sig.		.051	.051	.218
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 3.000.				

FI		
Duncan		
Groups	N	Subset for alpha = 0.05
		1
p25	3	52.9833
p5	3	53.8100
positive control	3	54.6633
negative control	3	56.0200
p35	3	58.0333
p15	3	58.8200
Sig.		.123
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 3.000.		

FCR			
Duncan			
Groups	N	Subset for alpha = 0.05	
		1	2
p5	3	1.2633	
positive control	3	1.2900	1.2900
negative control	3	1.3467	1.3467
p15	3	1.4933	1.4933
p25	3	1.5067	1.5067
p35	3		1.5933

Sig.		.117	.057
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

FER		
Duncan		
Groups	N	Subset for alpha = 0.05
		1
p35	3	.6267
p25	3	.6667
p15	3	.6733
negative control	3	.7500
positive control	3	.7967
p5	3	.8000
Sig.		.053
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 3.000.		

PER		
Duncan		
gruops	N	Subset for alpha = 0.05
		1
P35	3	1.9597
P25	3	2.0797
P15	3	2.0968
NC	3	2.3491
PC	3	2.4900
P5	3	2.5009
Sig.		.055
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 3.000.		

CF			
Duncan			
Groups	N	Subset for alpha = 0.05	
		1	2
p5	3	.8867	

p25	3	.8900	
negative control	3	.9100	.9100
p15	3	.9400	.9400
p35	3	.9633	.9633
positive control	3		1.0400
Sig.		.246	.057
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

VSI		
Duncan		
Groups	N	Subset for alpha = 0.05
		1
p25	3	10.2100
p15	3	10.3200
p5	3	10.6967
negative control	3	11.4367
p35	3	11.6033
positive control	3	15.7200
Sig.		.074
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 3.000.		

HSI			
Duncan			
Groups	N	Subset for alpha = 0.05	
		1	2
p35	3	1.3667	
p25	3	1.6067	1.6067
negative control	3	1.8867	1.8867
positive control	3	2.0333	2.0333
p15	3	2.1833	2.1833
p5	3		2.4833
Sig.		.122	.099
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

GSI		
Duncan		
Groups	N	Subset for alpha = 0.05
		1
negative control	3	1.0400
p35	3	1.2667
p15	3	1.3833

p5	3	1.4433
p25	3	1.7200
positive control	3	2.0267
Sig.		.163
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 3.000.		

ANOVA results for sex ratio

Male Percentage					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	995.778	5	199.156	.858	.536
Within Groups	2786.667	12	232.222		
Total	3782.444	17			

Male Percentage		
Duncan		
Treatment	N	Subset for alpha = 0.05
		1
NC	3	62.67
P5	3	69.33
P25	3	70.67
PC	3	80.67
P15	3	82.00
P35	3	82.00
Sig.		.185
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 3.000.		