INFLUENCE OF DIETARY SOYA BEANS (*GLYCINE MAX* (L.) MERR.) PROTEIN AND LIPID COMBINATION, AND ANDROGEN (17 α – METHYL TESTOSTERONE) LEVELS ON THE GROWTH AND REPRODUCTION OF *OREOCHROMIS ANDERSONII* (CASTELNAU, 1861)

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DECLARATION

I Alexander Shula Kefi, declare that this thesis is a result of my own original effort and work, and that to the best of my knowledge, the findings have never been previously presented to the University of Malawi or elsewhere for the award of a PhD. Where assistance was sought, it has been accordingly acknowledged.

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Signature:----------

Date:----------

CERTIFICATE OF APPROVAL

We, the undersigned, certify that this thesis is a result of the authors's work, and that to the best of our knowledge, it has not been submitted for any other academic qualification within the University of Malawi or elsewhere. The thesis is acceptable in form and content, and that satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on 6th March 2014.

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DEDICATION

To all my family members who encouraged and missed me during the course of study.

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ABSTRACT

In order to investigate the influence of dietary soya bean protein and lipid combination, and androgen (17 α – methyl testosterone (MT)) levels on the growth, reproduction and sex ratios of *Oreochromis andersonii* (Castelnau, 1861), four experiments were conducted. In the first and second experiments, the fish were reared in 40 hapas for 55 days set in a Randomised Complete Block Design (RCBD) factorial structure with three levels of lipid and protein; 10%, 15% and 20% lipid, and 20%, 30% and 40% protein respectively. In the third and fourth experiments, fish were subjected to isonitrogenous (30%) and isocaloric (4.02 kcal/g) diets incorporated with three levels of MT at 40 mg/kg, 60 mg/kg and 90 mg/kg of feed laid in a Complete Random Design (CRD).

Growth performance of *O. andersonii* was significantly affected ($P< 0.05$) by different combinations of dietary protein and lipid with the best final weight and body weight gain (BWG) achieved at 20% crude lipid and 40% crude protein combination although not significantly different ($P> 0.05$) from any combinations with 30% and 40% crude protein. There were no significant differences ($P> 0.05$) in the fish growth indices among the lipid levels used although polynomial regression showed 15.3% to have given the highest body weight gain. Gross margin (GM) and total cost (TC) were highest too at the highest lipid and protein combinations. This also shows that protein level may be more important in fish feeds than lipid level. The study showed that fecundity and Gonadosomatic Index (GSI) were maximized at 15% crude lipid and 20% crude protein while the egg size was maximized at the 15% crude lipid and 40% crude protein combinations. However, the number of eggs incubated was maximized at the highest protein (40%) and lipid (20%)

levels used in the experiment. The study showed that female *O. andersonii* can spawn without the presence of males and mature earlier than males. Fast growing fish were more likely to mature earlier than slow growing ones. The study revealed that the growth of *O. andersonii* was maximized when the feed was incorporated with the 60 mgMT/kg although the highest GM was observed in the control group (without MT). The GSI, Gonadal Index (GI) and egg size were not significantly different ($P > 0.05$) among the MT levels. This was also confirmed by the functional gonads as observed by the histological examination of the gonads. The anabolic effect achieved during oral administration of the androgen in the first 30 days of life declined with time. The study showed that the oral administration of MT does not affect the physiology of *O. andersonii* as the haematology chemistry and histopathology of the liver and heart were similar ($P > 0.05$) to that of the control. The 60 mgMT/kg feed achieved 94.4% males although this was not significantly different ($P > 0.05$) from the 93.4% males produced by the 40 mgMT/kg feed treatment. However, males were significantly skewed ($P < 0.05$) from the control group (54.3%) and the 90 mgMT/kg feed (79.3%).

In conclusion, the studies suggest a combination of 30% crude protein and 10% crude lipid sourced from soya bean and maize in the culture of *O. andersonii* but optimised at higher than 40% for protein and at 15.3% for lipid for growth. Egg size is maximised at a combination of 40% crude lipid and 15 % crude lipid a combination recommended for brood stock. The studies further suggest a 60 mgMT/kg feed in the sex – reversal of *O. andersonii.*

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Global aquaculture production

Fisheries and aquaculture directly and indirectly play an important role in the livelihoods of people around the world as the source of income and food. In 2008 it was estimated that 44.9 million people were involved directly or indirectly and of these 12% were females (Food and Agriculture Organisation [FAO], 2010). The sector attracts the world's employment above the human population and in traditional agriculture (FAO, 2010). Both capture fisheries and aquaculture supplied the world 130.8 million tonnes of food fish in 2011 of which 46% was attributed to aquaculture providing an apparent per capita consumption of 18.8kg which is all time high (FAO, 2012). Overall fish provided 2.9 billion people with at least 15% of their average per capita consumption of animal protein. According to Tacon and Foster (2001) an increase of 50 million tonnes of fish will be needed by 2050 to meet the ever increasing human population estimated to reach 8.32 billion by 2030. Since capture fisheries has reached its maximum potential (FAO, 2004; 2010) which stopped to grow in the mid - 1980s (FAO, 2009), aquaculture remains the only option to meet this demand (Figure 1.1).

Between 1970 and 2008 aquaculture contribution to the total fish supply per capita grew from 0.7kg to 7.8kg outpacing the human population growth with total production of 52.5 million tonnes valued at US$ 98.4 billion (FAO, 2010). This represents an annual growth rate of 6.6% making aquaculture the fastest growing food production sector in the aforementioned period. The growth is above the human population growth rate although

the per capita fish food supply remains constant (Figure 1.2). By far, countries in Asia and the Pacific regions accounted for 89% of total production and 77% in terms of value (FAO, 2010).

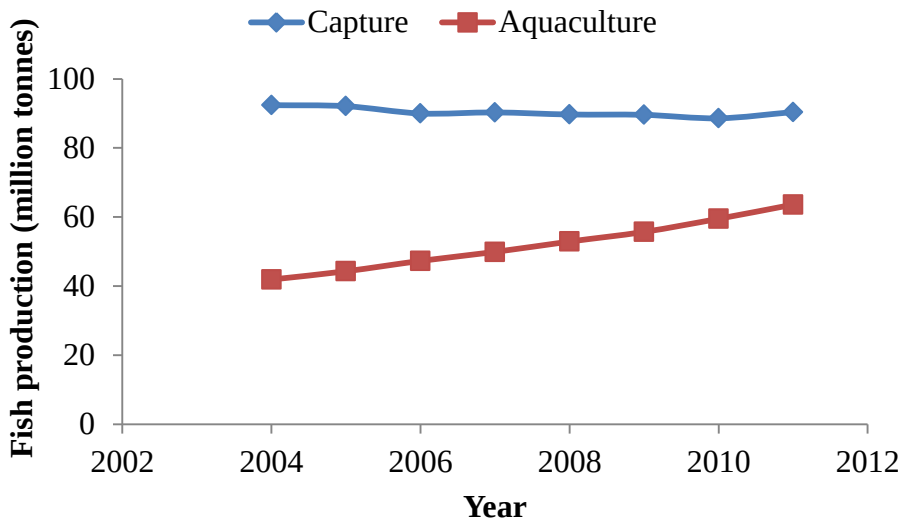


Figure 1.1: Trend of fish production from both aquaculture and capture fisheries (Source: FAO, 2010; 2012)

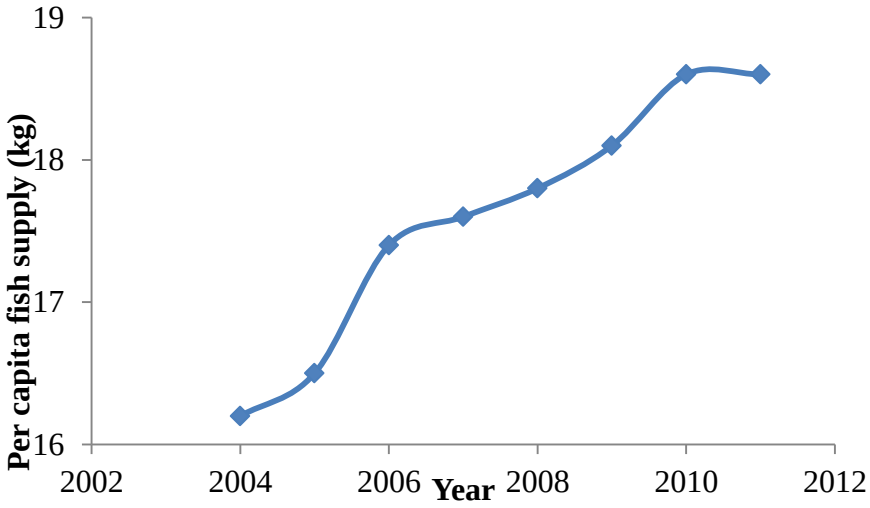


Figure 1.2: Global per capita fish food supply (Source: FAO, 2010; 2012)

China alone contributed 47.5 million tonnes (32.7 and 14.8 million tonnes from capture fisheries and aquaculture, respectively) in 2008 despite a downward trend report for both capture fisheries and aquaculture by 10% corresponding to 3 million tonnes in aquaculture in response to the China's 2006 National Agriculture census which questioned fish production figures (FAO, 2012).

In 2010 Africa contributed 2.2% to the total world's aquaculture production despite the huge potential with Egypt leading the production of 919, 585 tonnes representing 71.4 % (FAO, 2012). However, there has been an increase of 56% between 2003 and 2007. This growth has been attributed to an increase in the prices of aquatic products and the expanded cage aquaculture in several African countries. The aquaculture production from Sub Saharan Africa still continues to produce below 1% mark although there has been an increase of 0.2% in 1970 to 0.5% in 2008. Catfish and tilapia are the main fish species with Nigeria leading at 85, 000 tonnes of the former (FAO, 2009).

1.2 Aquaculture in Zambia

Fish farming in Zambia started in the 1940s when the indigenous fish species of the family Cichlidae were introduced in dams and earthen ponds mainly after the construction of six fish ponds in 1943 at Chilanga Fish Farm located 15km south of the capital Lusaka. Later the Government established a fish farm at Mwekera now the National Aquaculture Research and Development Centre (NARDC) in 1950s which is 25km East of Kitwe town the third largest city in Zambia. This was done in order to demonstrate the establishment of fish farming ventures and supplement the experiments

that were being conducted at Chilanga (Utsugi and Mazingaliwa, 2002). At that time the emphasis was subsistence farming as opposed to viewing aquaculture as a business. However, aquaculture stagnated for more than twenty years (1966 – 1986) partially because the policy favoured capture fisheries as this involved more people. The policy ignored the fact that fish farming could overcome the low supply of fish from the natural water bodies (Aquaculture Development Plan [ADP], 2009). Later government established seed production centres across the country to try and stimulate fish farming. A total of 19 fish farms have been established across the country in all the ten Provinces. However, due to low funding and lack of well-motivated staff, most of these are functioning below capacity.

There are more than 6, 000 small – scale fish farmers owning over 13, 000 fish ponds throughout the country (ADP, 2009). Commercially, there are also active large commercial fish farms located in the high densely populated Provinces of Lusaka and Copperbelt where the income levels are high. The Southern Province has also seen development in cage farming in Lake Kariba culturing the exotic *Oreochromis niloticus*. It is estimated that a total of 12, 988 tonnes of fish was produced in 2012 (Department of Fisheries [DOF], 2013).

Fish consumption has been declining over the years although the number of fish ponds has gradually increased. This is attributed to the increase in the human population above the fish production increase due to low yields. In the early 1970s per capita fish consumption in the country was estimated at 17kg but gradually reduced as imports were

cut to help ease balance of payment problems. By the end of the decade per capita fish consumption had declined to 10 kg. However, this level of consumption was unevenly distributed between rural areas (7kg) and urban areas (15kg). It was clear the level of fish consumption could not be sustained given that maximum sustainable production from existing natural fisheries resources was only 74, 000 tonnes per year and the country was experiencing a rapid population growth (ADP, 2009). Based on the calculations from the current fish production it is estimated that the current fish consumption per capita stands at 5.8kg.

Zambia is richly endowed with natural resources ideally suited to aquaculture production. It is among the largest aquaculture producers in sub – Saharan Africa with an estimated production of just of 13, 000 tonnes in 2012 (DOF, 2013). Several reasons that derail aquaculture production in Zambia include, but not exhaustively, predation and thefts, unavailability of fish seed in sufficient numbers and of good quality, poor communication facilities and lack of capacity to use new technologies among the small scale farmers (Japan International Cooperation Ageny [JICA], 2008). These bottlenecks have resulted in the fluctuations of fish production as farmers tend to abandon and later restart the operations when resources are available (FAO, 2009). To improve the quality and quantity of the offspring that ensures maximum survival and fast growth, understanding brood stock nutrition, therefore, becomes indispensable. This is because many of the deficiencies and problems encountered during the early rearing phases of newly hatched finfish larvae are widely related to the feeding regime of the brood stock (Izquierdo *et al*., 2001). It is possible and expected that the quality and performance of larvae may also be

affected by the female's dietary regime because, until the onset of first feeding, larvae are solely dependent on the nutrients supplied by the yolk (Gunasekera *et al*., 1996a). Due to increased interests in aquaculture production, calls and efforts have been concentrated on seed quantity rather than quality. This has not helped as fish production still remains low and introduction of exotic fish species seems to be growing due to lack of confidence in indigenous fish species.

In Zambia, the indigenous *Oreochromis andersonii*, *O. macrochir* and *Tilapia rendalli* are the leading aquaculture fish species as they are readily acceptable to the consumers. However, the exotic *O. niloticus* is steadily gaining popularity among the farmers across the country as it is believed to have superior growth rate compared to the indigenous fish species (Mudenda, 2004). Almost Fifty percent of the aquaculture production in 2012 was attributed to *O. niloticus* (DOF, 2013). The species was imported to Zambia in 1982 from University of Stirling basically for farming at Zambia Sugar Company (ZSC) since at that time Zambia had a liberal policy with respect to fish translocations and introductions. It was cultured on a large scale by 1990 due to its superiority in resisting stress compared to indigenous fish species (Schwanck, 2004). The invasion of the natural system in Zambia by this fish species might have occurred in 1987 and by 1990 its presence was reported 75km from the point of release (Schwanck, 2004) in Kafue River, a major tributary of Zambezi River (Howard, 2004). At present the fish is widely distributed in the Kafue River basin and is locally known as *Wamunyima* named after the then Manager at ZSC. It is also known as *Ma dam* referring to fish found in reservoirs.

Bbole (2012) showed evidence of hybridisation between *O. niloticus* and indigenous fish species with a strong interaction found with *O. andersonii*.

The fish was introduced in Lake Kariba basically for cage culture and across the country for small scale farming mainly by Pearce Corps Zambia a United States of America (USA) volunteer organization engaged in aquaculture extension programmes. In October 1980, the Government of Republic of Zambia (GRZ) through the Department of Fisheries (DOF) imported 200 pieces of Mirror Carp (*Cyprinus carpio carpio*) fingerlings from Domasi station in Zomba now called the National Aquaculture Centre (NAC), Malawi and introduced them at Chilanga Fish Culture Station (Soma *et al*., 1997). At around the same time, the same fish culture station imported Scaled Carp (*Cyprinus carpio*) from Czech Republic then Czechoslovakia. Due to poor water quality at Chilanga a decision was made to transfer some carp fish to Mwekera Fish Farm in Kitwe, Copperbelt Province where the culture and propagation has continued up to date. However, its propagation has declined over the recent past as farmers still prefer Tilapia species.

It is important though to note that alien genotypes have been recognized as one of the major threats to aquatic biodiversity (Bartley and Marttin, 2004). Being exotic, their effect on the indigenous species may be detrimental ecologically (Lowe - McConnell, 1987). Therefore, *O. niloticus* and *C. carpio* cannot be ignored but must be treated carefully.

1.3 *Oreochromis* species

Originated from Africa and the Middle East (Fryer and Iles, 1972), tilapia is a name used for a large number of species within the cichlid tribe Tilapine (Beveridge and McAndrew, 2000) and is the most important warm water fish grown for fish production (Charo – Karisa, 2006). Tilapias are one of the fastest grown cultured fish species in the world with an average growth rate of 13.4% in the 1970 – 2002 (El – Sayed *et al.*, 2005) and are widely farmed and dominate aquaculture production in Africa (Jamu and Ayinla, 2003). Their use in aquaculture spread throughout Africa after the 1920s and became established as potential farmed fish species in North America in the 1950s (Iversen, 1976). Of this group it includes the fish species of the genera *Tilapia*, *Sarotherodon* and *Oreochromis*. In terms of the breeding habits, *Tilapia* is a substrate spawner with both parents guarding the spawn in the nests. The species in the genus *Sarotherodon* exhibit biparental or paternal mouth brooding of the eggs where both the males and females guard the spawn or the males only providing the parental care. *Oreochromis* species are named for their maternal mouth brooding behaviour. The mouth brooding aspect is an important characteristic in aquaculture as the spawn can be collected from the mouth of the fish for possible hatching control and sex manipulation.

Compared to other fish species such as catfish, *Oreochromis* species exhibit low fecundity that may mean keeping large numbers of brood fish (Ambali and Little, 1996), if reasonable quantities of fish seed for stocking in fish ponds are to be produced. However, low fecundity assures high survival of the offspring (Moyle and Cech, 2002). Furthermore, its ability to reach sexual maturity at an early age usually before the market

size poses another challenge. Early sexual maturity may have a negative effect on the growth rate leading to a phenomenon called stunting thus fewer marketable size fish (de Graaf *et al.*, 1999; Peña – Mendoza *et al.*, 2005). Given that *Oreochromis* species reared are mouth brooders with low fecundity and sluggish initial growth rates, there is every need to explore their specific reproductive biology starting from the brood stock nutrition point of view as a way of exploiting ways of manipulating their reproductive and growth potential.

1.3.1 *Oreochromis andersonii* (Castelnau, 1861)

In the wild, *O. andersonii* is found naturally in lagoons of the Upper and Middle Zambezi River, Kafue systems (Skelton, 2001) and Lake Kariba (FishBase, 2009). Adults occupy deep water while juveniles remain inshore. It feeds on detritus and zooplankton although bigger individuals also take insects and other invertebrates. It changes the diet according to the food availability making it a suitable fish for aquaculture (Gopalakrishnan, 1988). In both the females and males, there are 3 or 4 spot like conspicuous mid – lateral blotches and a red margin on dorsal and caudal fins thus the name 'three spotted bream' (FishBase, 2009).

The male genital papilla is bluntly conical with a narrow flange slightly notched in the middle with jaws enlarged in breeding males. The males make saucer-shaped depression nest where the female deposits the eggs. The female broods the eggs in the mouth (FishBase, 2009). In pond condition, a female *O. andersonii* is able to incubate on average 639 eggs with the maximum number counted 2, 300 (Kefi *et al.*, 2011).

Figure 1.3: *Oreochromis andersonii* nest taken in an earthen pond at NARDC, Zambia

The fish has dorsal spines ranging between 15 – 18 dorsal soft rays: 11 – 15; 3 anal spines; anal soft rays: 9 – 13; vertebrae: 30 – 32; the scales in the lateral line series vary between 31 – 35, but usually 32 – 33. The total number of dorsal rays is 28 – 31. Body depth may be 40.5 – 50.5% of the Standard Length (SL) (FishBase, 2009).

Figure 1.4: *Oreochromis andersonii* (male (top) and female) taken at NARDC, Zambia

The popularity of *O. andersonii* in fish farms might have emanated from the trials of 1980 – 1982 conducted at Chilanga Fish Farm, which indicated its suitability in integrated fish farming (Gopalakrishnan, 1988). It exhibited tolerance to cold temperature and handling, and showed high growth rates compared to other local tilapia species under semi-intensive system. The fish prefers slow – moving or standing water probably a reason why it adapts well in pond conditions (Skelton, 2001).

1.4 Problem Statement and Justification of the Study

Zambia is a land locked country, surrounded by eight neighbouring countries, namely, Angola, Botswana, Democratic Republic of Congo (DRC), Malawi, Mozambique, Namibia, Tanzania and Zimbabwe with an estimated total population of 13, 046, 508. Of

this population the majority (61 per cent) live in rural areas. The population is skewed towards females at 51% (CSO, 2011). The country has a relatively large land surface area of 752,672 km^2 which, lies on the Central African high Plateau at an average altitude of 1 200m above sea level. This elevation modifies temperatures which are lower than the coastal areas at the same latitude. Zambia experiences a dry, hot and wet season from September to April with air temperatures ranging from 17°C to 35°C while the cold season starts from May to August which is cold but dry with air temperature ranging from 6°C to 26°C. Water temperature ranges between 15°C and 22°C in cold season and 22°C and 30°C in the hot season. This poses a challenge to fish production as growth and reproduction are depressed in cold months since the most preferred fish species for culture are Tilapias whose optimal temperature for growth and reproduction fall within 25°C– 30°C (Bocek, 2006).

The poverty in the country stands at least 64 % of the population of which the rural poor are the majority (CSO, 2011). This means that the immediate challenge of the country is to reduce hunger. This is because reducing hunger directly reduces poverty in the long run. Aquaculture is able to offset some severe forms of malnutrition and poverty, and can contribute directly to the achievement of Millennium Development Goals. This can be achieved due to the availability of 29, 000 ha of land that has been identified as suitable site for fish production (ADP, 2009).

Figure 1.5: Map of Zambia showing all the eight neighbours (Source: http://www.google.com/index.html).

Broodstock nutrition is an important aspect in fish breeding and reproduction and therefore, essential in the production of quality fish seed. Mohan (2007) defined fish seed quality as that which benefits the hatcheries, nurseries, intermediaries in the seed supply network and finally the producers. This is because the dietary requirement of the brood stock is different from that of juvenile or other stages of fish. Seed in fish can be eggs, milt, fry, fingerlings or nursed fish (Bondad – Reantaso, 2002). Mohan (2007)

described seed quality as a fundamental pre - requisite for sustainable and successful aquaculture. It is active, healthy in appearance, uniform in size, potential for high growth, better growth, less or no disease. Therefore, management of brood stock has critical impacts on the health status and subsequent performance of seed (Mair, 2002; Mohan, 2007). In brood stock nutrition the most important aspect is the quality of larvae. An improvement in brood stock nutrition has shown to improve not only egg and sperm quality but also seed production (Izquierdo *et al*., 2001). The energy utilization to the various physiological processes of the parent fish affect the size, quality and quantity of eggs produced (De Silva and Anderson, 1995). In continuous spawners such as *Oreochromis* species, gonadal development and fecundity are affected by certain essential dietary nutrients. Therefore, effective seed production demands a good understanding and in particular of brood stock nutrition which significantly affect fecundity, survival, egg size and quality which consequently affect the larval quality (Bromage, 1998; Watanabe and Vassalo – Agius, 2003). This is important for planning and management of the brood stock facilities, particularly due to the costly installations required for the *Oreochromis* species with low fecundity.

There are important effects of nutritional manipulations that affect egg quality. Okumura *et al*. (2002) speculated that low fertilization in the red spotted grouper, *Epinephelus akaara*, would be attributed to poor nutrition and stress under high densities in culture facilities. Gunasekera *et al*. (1996a) found that protein level in the diet affected the protein level in the eggs of *O. niloticus*. Lipid and fatty acids composition of brood stock diet have been identified as major dietary factors that determine successful reproduction

and survival of offspring in some fish species. Fecundity was found to increase in rabbit fish (Duray *et al*., 1994), seabream (Fernández – Palacios *et al*., 1995) and sparids (Watanabe *et al*., 1984) after they were fed with diets with lipid and fatty acids. Rainbow trout fed with essential fatty acid deficient diet produced low eyed eggs with low hatchability (Watanabe *et al*., 1984). They also found that female Red Sea Bream that received a diet with low protein and deficient in phosphorus and essential fatty acid produced eggs with low hatchability and the larvae that hatched had body deformities.

There are few published reports on the influence of the fish female's nutritional history on the progeny of the fish, particularly in warm water species (Gunasekera *et al*., 1996b). Although attempts have been made (Watanabe, 1982; Santiago and Reyes, 1993) to investigate the effect of dietary lipids on the reproduction of *O. niloticus*, emphasis has been on the substitution of one type of lipid source for the other rather than on the lipid level. Where the influence of varying dietary lipids has been done (Chou and Shiau, 1996) emphasis has been on its effect on the growth rather than reproduction. In addition, the studies have been single laboratory experiments rather than in combination and so their applicability at farm level has been limited since they cannot explain complicated biological systems where many factors are interacting. Since the primary source of nonprotein energy in fish is lipid the diets can be reported as the ration of lipid to protein (Lovell, 1998).

Gunasekera *et al*. (1996a, b) and Khan *et al*. (2005) studied the effect of dietary protein level on the spawning and larval quality in *O. niloticus* and *Labeo rohita*, respectively.

The sparing effect of proteins by lipid has been studied in salmonids, trout and some selected Tilapias (Guillaume *et al*., 2001). In Salmonids it has been estimated that an increase in lipid level from 15% to 20% allows the protein content to be lowered from 48% to 35% without altering growth performances. It has been found that in trout increasing the lipid content from 14% to 20% improves growth and feed efficiency even when protein content is decreased. No similar studies have been reported in *O. andersonii*, a species of particular interest in Zambian fish farming sector. As such nutritional requirement for the *O. andersonii* brood stock is not well understood.

The accumulation of yolk in fish eggs during their development is important for proper embryonic development after fertilization and is a key process for every successful reproduction (Levi *et al*., 2009). This process referred to as vitellogenesis begins after the production of the follicle stimulating hormone (FSH) in the pituitary which in turn stimulates the production of the estradiol (estrogen) in the ovarian follicles. Estrogen in turn induces the liver to produce vitellogenins (VTG) which are egg yolk precursors. Lokman *et al*. (2007) reported that androgens do serve as precursors for estrogen synthesis and have a functional role during previtellogenesis in teleost fish in general. They implicated the possible functional role of androgens in the control of previtellogenic oocyte growth in eel. Prior to previtellogenesis, androgens are suggested to play an integral role in the regulation of oocyte growth and development (Kortner and Arukwe, 2008). Based on the findings of Lokman *et al*. (2007) *in vitro* studies on short finned eel revealed that at least the effects of 11 – KT may be direct.

The testes of fish synthesize testosterone, androstenedione and a potent androgen 11 – KT (Gazola and Borella, 1997). A study done by Rohr *et al*. (2001) revealed that prolonged treatment with the androgen 11 – KT *in vivo* was found to increase the size of previtellogenic oocytes in short fined eel, *Anguilla autralis* while estradiol did not (Lokman *et al*., 2007). However, it still remains unclear what endocrine and/or intraovarian factors that regulate oocyte growth and how they may influence the timing of puberty, fecundity, egg quality and early embryogenesis. Very little work has been done on the endocrine effect on egg size and consequently batch size. The roles or effects of androgens on the ovary of the fish have not been described before (Kortner and Arukwe, 2008).

This study investigated the effect of varying the amounts of soya bean protein, lipid and the incorporation of the androgen MT into the brood stock feed administered to *O. andersonii* brood stock on fecundity, size and quality of the eggs and their influence on the growth of the juvenile *O. andersonii*. Costs and returns of producing diets with varying levels of protein and lipid sourced from soya beans were investigated too. Furthermore, the efficacy of MT on sex reversal of *O. andersonii* was investigated.

1.5 Statement of objectives

1.5.1 Main objective of the study

- To investigate the effect of different combinations of soya bean protein and lipid, and MT on the growth, fecundity and egg size and the influence of MT on the sex ratios and growth of juvenile *O. andersonii.*

1.5.2 Specific objectives

- To determine the optimal combination of dietary soya bean protein and lipid for growth and its effect on whole body composition of *O. andersonii*.
- To determine the effect of dietary soya bean protein and lipid combination on sexual maturity, fecundity, egg size and survival of *O. andersonii*.
- To determine the effect of MT on the growth, fecundity, sexual maturity, survival, egg size, haematology, liver and heart of *O. andersonii*.
- To determine the effect of administering different levels of androgen MT on the sex ratios of *O. andersonii*.

1.6 Null hypotheses

- **Ho_1:** Dietary soya bean protein and lipid combination levels do not have any effect on growth and whole body composition of *O. andersonii.*
- **Ho_2:** Sexual maturity, fecundity and egg size of *O. andersonii* are not affected by different combinations of lipid and protein.
- **Ho_3:** 17 α – methyl testosterone has no effect on growth, sexual maturity, fecundity, egg size, haematology, liver and heart of *O. andersonii*.
- **Ho_4:** The sex ratios of *O. andersonii* administered with different levels of MT are the same.

1.7 Structure of the thesis

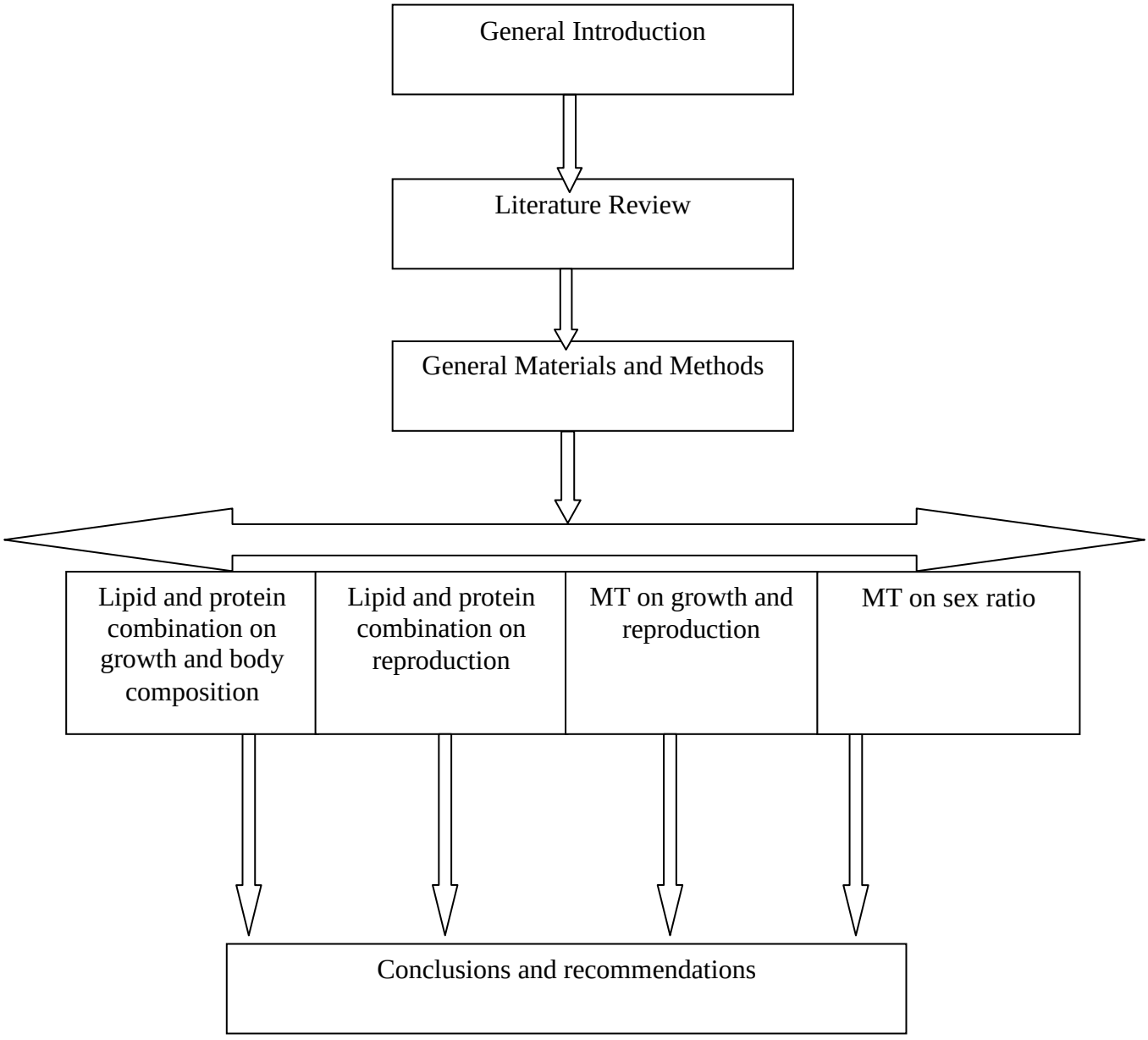


Figure 1.6: Schematic diagram showing the structure of the thesis

CHAPTER 2

LITERATURE REVIEW

Although indigenous Tilapia species culture is very important in Zambia, little research work has been done on indigenous fish species to enhance their production. Department of Fisheries (DOF) has chosen *O. andersonii* as the indigenous species for promotion in the Zambian aquaculture. Literature on the effect of lipid and protein on reproduction and growth on fish has been reviewed. Furthermore, hormonal utilization review on growth, reproduction and sex reversal of fish has been equally presented.

2.1 Factors that affect fish growth

Major characteristics that determines the suitability of a fish species for aquaculture is the rate of growth and production under culture conditions. The factors that determine the rate of growth in fish can be divided into exogenous factors imposed by the environment and endogenous ones related to the genotype, body size and physiological condition of the fish (Wootton, 1998).

In exogenous factors, fish growth is a function of feeding, food utilization, presence or absence of stressing factors (in particular water quality), and hierarchical behaviour and that these bear a positive relationship with temperature (Jobling, 1995). Fish are poikilothermic animals, that is, their body temperature is the same as, or 0.5 to 1°C above or below, the temperature of the water in which they live. The metabolic rate of warm water fish is closely correlated to the water temperature: the higher the water temperature (i.e. the closer to the optimum values within the normal range), the greater the

metabolism. In addition, temperature greatly influences their ability to feed, utilization of the consumed food, cell division and differentiation and hence growth (Jobling, 1995). Bocek (2006) reported that the optimal growth temperature for tilapias ranges from 25 to 30^{o}C.

The analysis of genetics of growth in fish is difficult due to the flexibility patterns in fish's growth and sensitivity of growth to myriad environmental influences including social interactions (Purdom, 1993; Wootton, 1998). The contribution of genetics to fish growth can only be measured indirectly through the fish's phenotype (Tave, 1990). An integral concept in genetic improvement through selection is heritability (Greg, 2001). Heritability gives an estimate of the contribution of genetic factors, relative to the environmental factors manifested by a phenotypic trait such as growth in a population at a given time (Wootton, 1998). Growth rate can be as a result of genes inherited from the parents. Therefore, management of genetic fish resources becomes important in maximising the full potential of fish growth.

2.2 Gonadal development and factors that affect egg quality in fish

Gonadal differentiation starts early in the development process of the fish's life. For instance in yellow perch, *Perca flavescens*, differentiation of gonads starts at the larval stage (Malison *et al*., 1986). This is after external or internal stimuli affect the brain by modifying the specific neurohormones and neurotransmitters in order to regulate the secretion rates of gonadotropin – releasing hormone (GnRH), dopamine from the hypothalamus and, the pituitary gonadotropin (GtH) the process which is under the dual

hypothalamic control. At the end of the hypothalamic – pituitary – gonadal axis, GtH stimulates gametogenesis and steroidogenesis by testes or ovary. Within the gonads, germ cells produce gametes, and somatic cells support, feed and regulate the development of germ cells (Lukšienė and Svedäng, 1997).

The vitellogenetic period, which marks the end of secondary growth, involves the vacuolation of the oocytes and yolk and fat accumulation. Circulating vitellogenin is absorbed by the oocytes and changes to yolk granules before it is deposited. The yolk material consists of protein, fats, lipids and some carbohydrates. Vitellogenin is produced by the liver in response to estradiol which in turn is secreted by the ovarian follicle cells in response to GtH action (Nagahama, 1983). During this process, the weight of the gonads increases significantly. By the end of this process the oocyte would have reached its maximum size.

Androgens have been implicated in oogenesis in monkeys and women in the growth of prenatal follicles. In the androgen treated rhesus monkeys the number of primordial follicles advancing to the pre – or small antral stage increased (Vendola *et al*., 1998; Lokman *et al*., 2007). Similarly, women treated with testosterone or suffering from androgen excess showed increased numbers of growing follicles (Vendola *et al*., 1998; Lokman *et al*., 2007). During previtellogenesis, there have been increased levels of androgens in a number of fish species. Lokman *et al*. (2007) clearly showed that the androgen had a positive effect on the growth of oocytes. Survival has been found to

favour big fry produced from bigger eggs (Purdon, 1993). Survival of gold fish vitellogenic oocytes treated with the testosterone prolonged (Remacle *et al*., 1976).

Gametogenesis is controlled by the endocrine system, which requires the involvement of the brain – pituitary – ovary axis (Lokman *et al*., 2003). Although very little is known about the factors that regulate the previtellogenic or primary growth phase, Lokman *et al*. (2007) found that treatment of ovarian fragments with 11 – KT resulted in a significant increase in the diameter of eel oocytes implying that the androgen can exert direct effects on eel ovary, resulting in the increase of the size of the previtellogenic oocytes. Furthermore, they speculated that androgens do not only serve as precursors for estrogen synthesis but they have a functional role in previtellogenesis in teleost fish in general. Similar results have been reported in other animals. For instance, Vendola *et al*. (1999) found that androgens promote the initiation of primordial follicle growth implicate oocyte – derived IGF – I in this activation process in the primate.

Some studies have shown evidence that body size is related to egg biomass, and there is significant positive relationship between body size and egg production (fecundity and egg size). Studying on Sockeye and Kokanee Salmon, *Oncorhynchus nerka*, Kaeriyama *et al*. (1995) concluded that although fecundity varies with body size according to environmental factors, egg size may be affected by both environmental and genetic components such as polygene model (where the trait is controlled by many loci) within a cohort or population. They further construed that for the hatchery released sockeye salmon egg size may strongly relate to the genetic component.

Many fish species tend to decrease their food intake during sexual maturation and the energy and nutrients needed for ovarian growth are taken from their body reserves (Lal and Singh, 1987). This is because as the fish approaches its maturity an increasing proportion of the retained energy is stored as fat. Reproduction involves the synthesis of and temporary storage of new material which is formed almost regardless of the level of dietary energy intake, necessary energy being withdrawn from other body tissues if the dietary supply is insufficient. Fish progeny quality is defined by the size, shape and transparency of the egg, buoyancy, number and distribution of lipid droplets, biochemical composition of the eggs, rate of fertilization, appearance of the chorion, cell symmetry, chromosome appearance, hatching rates, larval survival rates and morphological deformities. Egg quality is a useful indicator of the viability and quality of the resultant larvae (Brooks *et al*., 1997). Factors affecting egg quality are determined by the intrinsic properties of the egg itself and the environment in which the egg is fertilized and subsequently incubated. The egg quality in fish is very variable. Some of the factors affecting egg quality in fish are known, but many (probably most) are unknown. Some of the known factors include the endocrine status of the female during the growth of the oocyte in the ovary, the diet of the brood fish, the complement of nutrients deposited into the oocyte, and the physiochemical conditions of the water in which the eggs are subsequently incubated (Suzanne *et al*., 2004).

Brood stock nutrition and feeding has been found to affect greatly the sperm and quality of seed production (Izquerdo *et al*., 2001). The fertilized eggs rely on the nutritional

components of the yolk as lipids, carbohydrates and proteins consumed prior to hatching (Hardy, 1985). The most relevant environmental parameter on fecundity is food, because the most critical transformation is food into progeny. A study on rainbow trout showed that ration size affected fecundity. In Atlantic cod, *Gadus morhua*, the amount of food only affected the repeat spawners while body size seemed to have affected fecundity of first spawners when the oocytes were accumulating the yolk (Wootton, 1998).

In many temperate and tropical fishes (referred to multiple, serial or heterochronal spawners) annual fecundity is seasonally indeterminate and batch fecundity is the only useful measurement. This is because the oocytes occur in nearly all maturity stages and so the standing stock of the yolked eggs gives no indication of the annual fecundity (Hunter *et al*., 1985). Predictable variations in batch fecundity could arise from genetic differences between females or from the effect of the environmental factors on oogenesis or from interaction between genetic and environmental factors (Wootton, 1998). Oogenesis is an integral aspect of reproduction that comprises of multi – step processes resulting in egg laying and can be subdivided into previtellogenesis, vitellogenesis and final oocyte maturation (Kortner and Arukwe, 2008). However, very few factors are known that control the growth phase of the oocytes despite being important in the governing fecundity. In fishes with indeterminate annual fecundity maturation of oocytes and vitellogenesis are a continuous cycle and proceeds rapidly after spawning after with ovary doubling in dry weight during the interval between spawnings (Hunter and Leong, 1981). The final stage of maturation or hydration is characterized by a rapid secretion of fluid of low specific gravity into the advanced eggs by the granulose cells of the follicle.

Hunter *et al*. (1985) itemized methods that could be used to identify oocytes to be included in the batch fecundity. The methods include 1) counting of all yolked oocytes and this is suitable for fish species that show determinate fecundity in a season; 2) estimation of the number of oocytes in the most advanced spawning batch by measuring the size distribution of oocytes in the ovary and identifying the most advanced group of oocytes and 3) estimation of the number of the oocytes in a spawning batch by counting the number of hydrated oocytes. They believed that the third method is preferable as it requires less time and avoids time to partition the oocytes between the most advanced mode and the adjacent group of smaller oocytes.

The main constituent of fish eggs are proteins (55 – 75%) and lipid (10 – 35 %) of dry weight (Kamler, 1992; Wootton, 1998). Studies on Threespine Stickleback, *Gasterosteus aculeatus*, by Fletcher and Wootton (1998) and on rainbow trout (Springate *et al*., 1985) showed that changes in dietary quantities had an insignificant effect on the chemical composition of eggs. Studying Red Sea Bream, *Pagrus major*, Watanabe *et al.* (1984) found that different compositions of the diet had little effect on the proteins, fat, water or minerals in the eggs, although the fatty acid content was similar to that in the feed.

2.3 Protein and amino acids (AAs)

The term protein come from a Greek word *proteios* that means of 'first importance' was named by the Johannes Mulder (Portugal and Cohen, 1977). They are a very important class of food molecules because they provide an organism with carbon and hydrogen and also with nitrogen and sulphur. The latter two are unavailable from lipids and

carbohydrates (Denniston and Topping, 2008). They are organic made of AAs arranged in a linear chain and folded into a globular or fibrous form. Therefore, AAs are the major building blocks for protein deposition and growth (Conceição *et al*., 2010). Like other biological macromolecules such as polysaccharides and nucleic acids, proteins are essential parts of organisms and participate in virtually every process within cells. They have many biological processes (Denniston and Topping, 2008).

Fish, like higher vertebrates and many invertebrates are incapable of synthesizing certain AAs and must be provided in the diet. These AAs are termed essential amino acids and these include Arginine, Histidine, Isoleucine, Leucine, Lysine, Threonine, Tryptophan, Valine, Methionine and Phenylalanine. Cysteine and tyrosine are considered as semi – essential amino acids since they are derived from the coupling of serine – methionine and phenylalanine, respectively (Guillame *et al.*, 2001). In fish proline and glutamine are semi – indispensable since their synthesis is slow. However, slow growth can be exhibited in fish if the synthesis of non – essential amino acids (NEAAs) (alanine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, proline, serine, cysteine and tyrosine) is slow, thereby, showing its essential character in nature. Gaye – Siesseger *et al.* (2007) found NEAAs to be important in the growth performance of *O. niloticus*. Proteins are the source of amino acids necessary for the biosynthesis during the early stages of embryogenesis (Conceição *et al*., 2010).

2.4 Lipid and fatty acids (FAs)

Lipids are a broad group of naturally fat – soluble compounds occurring in the tissues of plants and animals. They consist of fats, waxes, sphingomyelins and sterols (Pillay, 1990). The main biological functions of lipids include energy storage, as structural components of membranes and as substrate carriers in enzymatic reactions (Mcdonald *et al*., 1987). They also function as precursors of steroid hormones and prostaglandins. In fish nutrition they improve the flavour and texture of the feed (Lim *et al*., 2009).

Fats are the fatty acid esters of glycerol and they are the principal form of energy storage. They are a subgroup of lipids called triglycerides. They contain more energy per unit than any other biological product (approximately 8.5Kcal of metabolisable energy). Phospholipids are the esters of fatty acids and phosphatic acid and they are the constituents of the lipoprotein complexes of biological membranes. They are the main components of cell membranes determining the hydrophobic or hydrophilic properties of the membrane surfaces. They contain phosphorus in addition to carbon hydrogen and oxygen. Eggs are the best animal sources while in plants soya beans contain good amounts (Mcdonald *et al*., 1987). Sphingomyelins are present in the brain and nerve tissue compounds. Waxes are the fatty acid esters of long-chain alcohols and can be metabolised for energy. They are usually solid at room temperature. Sterols are polycyclic, long – chain alcohols and are components of several hormone systems especially those related to sexual maturation and reproductive functions, therefore, they are the precursors of the prostaglandins and steroids (Malcolm *et al*., 2002).

Fatty acids are described as saturated when they do not contain a double bond, and unsaturated when they contain one (mono – unsaturated) or more saturated (polyunsaturated) (PUFA) double bonds. Examples of saturated fatty acids include butyric, caproic, caprylic, capric, lauric, mysristic, palmitic, stearic and arachidic acid while PUFA include palmitoleic, oleic, linoleic, linolenic and arachidonic acids (Mcdonald *et al*., 1987).

2.5 Soya bean as the source of protein and lipid for fish feeds

Due to increasing demand, low supply and high cost of the most widely used animal protein fish meal; efforts have been directed in finding suitable substitute (Rinchard *et al*., 2002). Fish meal also causes phosphorus pollution (Phromkunthong and Udom, 2008; Cahu *et al*., 2009; Koumi *et al.*, 2011). Several plant and animal based sources have been suggested though the latter is expensive. For instance in Zambia in 2011 the cost of fish meal was US$1.5 against the US$0.6 per kilogramme of soya bean meal. Soya bean meal is used most extensively as a plant source of protein, especially in human and many animal diets due to its good nutritional and commercial value. Although its protein content (38 – 49%) is lower than that of fish meal (65%) its AA profile with the exception of methionine is considered to meet requirements for some of the fish species such as Channel catfish *Ictalurus punctatus*, Chinook Salmon *Oncorhynchus tshawytscha* (NRC, 1993) and gilhead sea bream *Sparus aurata* (Deguara, 1998). It has also relatively high amounts of vitamins (thiamine, niacin, B – Complex and carotene) (Martin and Ruberte, 1980; Fafioye *et al*., 2005).

In a study done at Bunda Fish farm in Malawi, Nyerenda *et al*. (2000) found that there were no significant differences in *Oreochromis karongae* fed on three diets of soya bean cake, fish meal and meat and bone meal. Similar results were found by Kapembwa (2009) on *T. rendalli*. However, the presence of anti-nutritional (substances which themselves or through their metabolic products arising in living systems, interfere with food utilisation thus affecting the health and production of animals (Makkar, 1993: Madalla, 2009)) factors such as trypsin inhibitors, phytates, lectins, phytoestrogens, antigenic compounds and antivitamin factors if not properly processed have depressed growth in fish. Probably this explains why several authors (Davis *et al*., 1989; Watanabe and Pongmaneerat, 1993; Stickney *et al*., 1996) have reported a negative relationship between the soya bean level and growth performance. However, these factors are heat labile and can easily be removed by heat treatment and extraction procedures. However, phytate is relatively heat stable and phytate bound P is not available to monogastric animals including fish (NRC, 1993; Abo – State *et al*., 2009). Competition as an ingredient with the livestock industry and for human consumption also possesses availability and accessibility challenges.

2.6 Effect of protein on fish growth and reproduction

Just like in other higher vertebrates there is an optimal level of protein for growth and reproduction of fish. However, the requirements for protein are very high in fish compared to other higher vertebrates. The optimal protein content of the fish diet is about twice higher than that for mammals or birds during the first few weeks of life (Guillaume *et al*., 2001). However, maintenance (replacement of worn out protein)

requirements or the quantity are much lower in fish for protein than in mammals. Furthermore, growth requirements or the quantity of food proteins taken for maintenance and synthesis of the body protein is in the same order of magnitude in fish and higher vertebrates. Therefore, the high amounts of protein in fish is derived from the fact that energy requirements are low resulting in low requirements for non-nitrogenous sources (energy sources) and thus in a high optimal dietary protein concentration (Guillaume *et al*., 2001).

The dietary proteins requirements for reproduction in fish are poorly understood although it has been accepted that the protein that ensure good growth also allow good synthesis of reproductive products in females and males. In the studies conducted by Gunasekera *et al*. (1995; 1996) found that the protein content influenced oocytes development and quality of eggs. The quality of female diet also plays an important role in the larval production, quality and performance. De Silva and Radampola (1990) found 30% crude protein as optimal for both males and females of *O. niloticus*. However, females that received feed with lower protein level spawned much earlier with higher relative fecundity and spawning frequency than those given a feed with a higher protein level. In an experiment conducted by Wee and Taun (1988) found that low levels of protein in the feed given to *O. niloticus* increased fecundity although the eggs were smaller than eggs produced by fish fed with higher levels of protein. In addition, low levels of protein increased spawning frequency although the onset of spawning was delayed.

2.7 Effect of lipid on growth and reproduction of fish

The protein component in fish feeds is the most expensive and must be kept to a minimum without compromising productivity. It has been proven that increasing the levels of lipid would reduce the protein level in fish feeds. In trout, increasing lipid levels from 14% to 20% improves growth and feed efficiency even when the protein content is decreased (Guillaume *et al*., 2001). Dietary lipids and their constituent fatty acids influence immune response and disease resistance in fish (Lim *et al*., 2008).

Among other constituents of brood stock diets, lipids have been found to greatly affect the composition of the eggs (Watanabe, 1985; Almansa *et al*., 1999). In addition, together with fatty acids in brood stock feed they have been isolated as major dietary factors that determine successful reproduction and survival of the offspring. Duray *et al*. (1994) found that elevation of dietary lipid levels from 12% to 18% in brood stock diets for Rabbit fish, *Siganus guttatus*, resulted in an increase in fecundity and hatching. Lokman *et al*. (2007) found that diameters of the oocytes increased after *in vitro* administration of 11 – KT with lipid supplementations.

A study done by Cavalli *et al*. (1999) on *Macrobrachium rosenbergii* found that eggs with higher *n* – 3 Highly Saturated fatty Acids (HUFA) had a better hatchability rate than those without the fatty acid. Watanabe and Vassalo – Agius (2003) found that in Red Sea Bream fish, diets with low levels of protein and deficient in fatty acids produced abnormal eggs of low hatchability and high larval deformities. Ali Hajizadeh *et al.* (2008) found that dietary lipid sources had no significant effect on egg diameter, egg

volume and egg dry weight of *O. niloticus*. However, relative fecundity was significantly different in fish fed with control diet.

2.8 Hormonal use in aquaculture

Sexual differentiation of gonads takes place in post hatching stage. The primodial germ cells at the time of hatching are found at the dorsal root of developing mesentery at mesoderm, ventral to the gut and in the endoderm cells of the gut in newly hatched fry before these cells migrate to the gonad region. The appearance of the ovocel and testocel an indication of sex differentiation to femaleness and maleness takes place between 30 – 33 days in case of *O. niloticus* (Mateen, 2007). This is an important period as this is the time when the undifferentiated cells respond to the exogenous and endogenous inducers of sex determination. Testosterone can be synthesized by both female and male gonads whereas oestradiol is produced exclusively by ovaries (Baroiller, 1988). Exogenous androgen causes complete or partial degeneration of female gonocytes. The gonads of genetically female fish may not be capable of producing estrogens for maintenance of oogenesis and the absence of the female hormone results in testicular development as an auto differentiation (Hackman and Reinboth, 1973).

Fish are sexually dimorphic to important production characteristics such as growth, survival, age at first sexual maturity, maximum size and external appearances (Donaldson *et al*., 1996). Tilapia males grow much faster than females. The European sea bass (*Dicentrarchus labrax*) shows skewed sex – ratio in favour of males under culture conditions, and their early maturation compared to females still represent important

drawbacks for commercial production (Zanuy *et al*., 2001; Menu *et al*., 2005). Production of the males becomes profitable in Tilapia culture while monosex culture of females in the sea bass, therefore, becomes preferable. Furthermore, Tilapia is precocious breeder maturing at an early age stage especially when food supply is limited resulting in overcrowding and hence low yield of fish harvestable size (Loya and Fishelson, 1969; Toguyeni *et al*., 1996).

There are several techniques that have been employed to produce monosex fish. These techniques include manual sexing (Guerrero, 1982), hybridization (Hickling, 1960: Ambali and Malekano, 2004), chromosome set manipulation (Ambali and Malekano, 2004), and hormonal sex reversal (Pillay, 1990). There has been interest too in sterilization as a method to ensure the use of genetically modified fish for aquaculture by preventing reproduction with conspecifics in an event of escapes. This is because intentional and non-international introductions may have irreversible implication on the ecology of natural systems. Production of monosex is possible because unlike in mammals and birds, fertilization in fish takes place externally and embryological development takes place without the protection of an internal womb or hard shelled egg. This has given an opportunity for aquaculturists to direct phenotypic sex by the addition of anabolic steroids hormones in feed or water. This is possible in that during early embryology, an embryo is, phenotypically, neither male nor female in that it does not possess ovaries and testes, therefore, it could develop into either a male or female (Tave, 1990).

The use of steroids have been described by several authors (Nakamura and Takahashi, 1985; Tayamen and Shelton, 1978; Goudi *et al*., 1983; Jae – Yoo *et al.,* 1988; Desprez *et al*., 2003) to be economical and thus common technique of producing monosex populations. Both androgens and estrogens have been used for sex inversion in fish. Steroids are called androgens if they are able to induce male characteristics and estrogens if they induce female characteristics. Androgens are steroid hormones derived from cholesterol synthesized by the adrenal cortex. Estrogens biosynthesis is mediated by the steroidogenic enzyme cytochrome P450 aromatase, which converts androgen to estrogen (Afonso *et al*., 2001). It is responsible for the development of the female reproductive tract and is produced in the ovaries.

Both types of the hormones have been used to sex reverse the fish phenotypically depending on the trait of interest. Some examples of androgens that have been used include: methyl testosterone, ethinyl testosterone (danazol), mibolerone, methyl dihydrotestosterone, hydroxyandrostenedione, dihydrotestostorone, testosterone acetate testosterone propionate and 11 – KT. Some used estrogens include stilboesterol, oestrone and ethynyloestradiol (Tave, 1990).

The supplementation of the anabolic steroid in the diet has been successfully applied for growth enhancement of many species (Donaldson *et al*., 1979; Higgs *et al*., 1982; Toguyeni *et al*., 1996). The *in vitro* administration of sex steroid have shown to stimulate appetite, increases food conversion ratio and modifies protein and lipid metabolisms (Matty, 1985; Hossain *et al*., 2002) although some research studies have proved

otherwise. Guerrero III and Guerrero (1997) found no apparent differences in the growth and survival of *O. niloticus* treated with androgen (androstenedione and MT) and that of control.

Use of androgens on sterile fish may restore masculinity but oestrogen treatment may fail to restore feminity (Mateen, 2007). Hormonal induction of sex reversal may also result in higher mortality among fish bearing homogamous (xx or zz) genotypes. However, estrogens have been used as effective growth promoters in cattle (Rumsey *et al*., 1999).

Several fish species exhibit reproductive dysfunction in captivity due to disruption of environmental cues. Some fishes such as *C. gariepinus* females fail to undergo final oocyte maturation, ovulation and spawning while males show low quality milt production, therefore, the need to induce ovulation and spermiation by the use of hormones, the technology that has been practiced since 1930s (Zohar and Mylonas, 2001).

The brain – hypothalamus – pituitary – gonad chain is the internal mechanism that regulates reproduction in fish. A hypothalamus is located at the base of the brain and controls many internal body functions including the activity of the pituitary gland and it produces gonadotropin releasing hormones (Jobling, 1995). It is sensitive to signals from sensory receptors and releases hormones in response to environmental cues. However, most of the fish species of economic importance fail to breed under captivity due to the disruption of natural environmental stimuli that occur naturally. For instance in Zambia

Tilapia, carp and catfish species fail to breed in cold season due to the low temperatures. Therefore, attempts have been made to obtain eggs of the highest weight possible and of the best quality in captivity or outside the breeding season by using hormones that accelerate the oocytes maturation and ovulation.

The use of hormones particularly synthetic ones has received a lot of criticism due to insufficient evidence on their ecological impacts (Baroiller and Toguyeni, 1996). Furthermore, consumers demand food that is free from hormones as such fish deemed to have been grown using hormones would fail to be sold and that industry could be destroyed by negative publicity (Tave, 1990). Production of animals using hormones for human consumption is still prohibited in many countries. However, several studies have shown that such fish would be free of hormones in the muscle. Teichert – Coddington *et al*. (2000) found that sex reversal of life early stage tilapia presents no negative impacts on human food safety. In that study regression analysis of available depletion data from tilapia showed that whole – body concentrations of MT and metabolites in tilapia attained of <100pg/g after 8 – 40 days of withdraw and that achievement of <100pg/g of MT and metabolites in carcass tissue occurs after 6 – 50 days of withdraw. The rapid dilution of effluent to very low levels in receiving waters, the sensitivity of MT to photo – oxidation, the expected rapid bacterial degradation and minute use of MT offers insignificant adverse environmental effects (Mateen, 2007).

2.8.1 17α –Methyl testosterone (MT)

The synthetic steroid MT is a male specific hormone (Rinchard *et al*., 1999) with affinity for the androgen receptor. In fish MT has been used for sex reversal or sex inversion especially in Tilapia at different concentration and administration methods. Dietary treatment and immersion are most acceptable methods for administering steroids although implants have been practiced too. There has been reported use of MT as reproductive hormone on some fish species. Lee and Tamaru (1988) used MT in the induction of maturation in male mullet. El – Greisy and Shaheen (2007) demonstrated that MT can bring male mullet to full reproductive capability without manipulating environmental conditions and therefore a potent spermiating agent although addition of cholesterol proved to be even more effective. Being synthetic, it is more potent than the natural androgens. Its potency is as a result of the presence of 17α – methyl group which makes its elimination slower than the natural androgens (Fagerlund and McBride, 1978; Donaldson *et al*., 1979; Baroiller and Toguyeni, 1996).

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Study area

The brood stock nutritional, reproductive and growth experiments of *O. andersonii* were conducted at NARDC (12°49'0" South and 28°12'0" East) in Kitwe, Zambia (Figure 3.1) the largest government owned aquaculture research centre in the country.

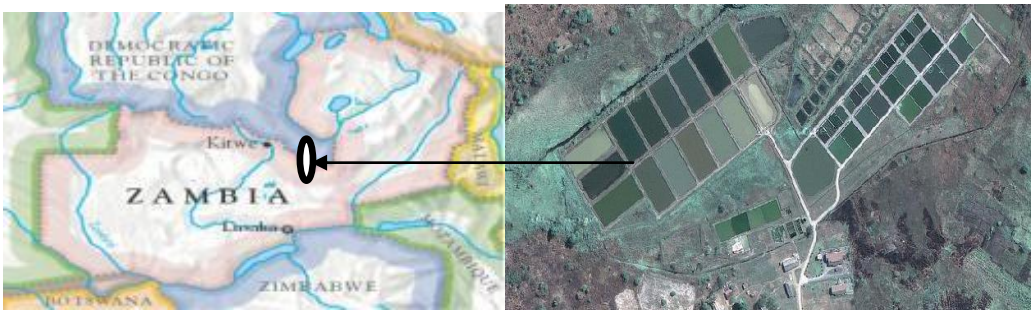


Figure 3.1: Study area (Source: http://www.maplandia.com/zambia/copperbelt/kitwe).

3.2 Experimental fish

The breeders (Table 3.1) were stocked in 2, 500m^2 earthen pond (Figure 3.2). Before the pond was stocked, it was prepared by removing the mud and cleaning the surrounding by slashing the grass on the dykes. A small trench of 10cm was then dug on the dyke around the pond. A black polythene sheet of 30 cm in height was then put around the pond on the dug trench supported by small bamboo sticks erected every 2m (Figure 3.2). Hydrated lime ($Ca(OH)_2$) was then sprinkled in and out of the polythene sheet at the rate of 0.1kg/m^2. The polythene sheet was put as barrier for the entry of predators such as frogs and otters. To determine the lime requirement of the pond nitrophenal method was

used to determine the pH of the pond mud. According to Hickling (1962) pond soils of pH above 7.0 would not require application of lime since they have a good buffering capacity. Accordingly, the pond was not applied with lime since the soil pH averaged 7.2.

Figure 3.2: Earthen pond used for breeding *O. andersonii* used in the experiment

Chicken manure (0.1kg/m^2) was then applied before allowing water to flow into the pond. After seven (7) days brood fish (males: 58.968 ± 28.550g; females: 47.555 ± 22.203g: mean ± SD) were stocked at 0.1fish/m^2 in 1: 2.8 male: female sex ratio (Table 3.1). Identification of sex was done manually by observing the genital papillae.

Table 3.1: Weight (g), standard and total length (mm) of the breeders

	Females	**Males**
	Mean ± SD	**Mean ± SD**
Weight (g)	47.555 ± 22.203	58.968± 28.556
Standard length (mm)	104.590 ± 16.143	114.419 ± 17.256
Total length (mm)	133.197 ± 20.095	145.048 ±20.413

Nests were seen in the pond two months after stocking. One month after observing the fry, a seine net was passed through to catch the juvenile fish. The collected fish were then anesthetized in crude clove powder (5g/L) (Unilever, South Africa) before they were sexed, weighed (g) and measured (SL and TL) according to Skelton (2001) and stocked in the pond cage (Figure 3.3). In the cage, fish were introduced to pelleted feed at 5% body weight twice a day (10:00 and 15:00hrs) with the 30% crude protein formulated using fish meal, maize bran, mineral premix and vitamin premix using WinFeed 2.8 version software (WinFeed (UK) Limited) after proximate analysis of the ingredients (AOAC, 2002). After two weeks, fish were scooped and transferred to the hapas set in semi – concrete ponds for the commencement of the experiments.

Figure 3.3: Pond cages used for holding fish before they were taken for experiments

3.3 Experimental feeds

The experimental feeds were formulated at NARDC following proximate analysis of ingredients. The ingredients included soya bean meal and maize bran which were locally acquired in Kitwe, Zambia. Other ingredients included in the feeds were pure soya bean oil SoyaGold oil (Thika, Kenya) which was extracted from soya beans, vitamin premix (AGRIMIX, Harare, Zimbabwe) and D – calcium phosphate (DCP) (Kitwe, Zambia). Nine experimental diets were formulated using WinFeed 2.8 version software (WinFeed (UK) Limited) (Table 3.2).

3.3.1 Proximate analysis of feed ingredients

Feed ingredients (soya bean and maize bran) were finely ground and passed through 1 mm mesh sieve. Chemical analyses of the feed ingredients were estimated in triplicate and average chemical analyses were calculated according to standard methods described by Association of Official Analytical Chemists (AOAC, 2002) to produce the nine experimental diets.

Table 3.2: The protein and lipid levels used in the experimental diets

Ingredients	Lipid (%)	Protein (%)	Code
No feeding	-	-	Unfed
Diet 1	10	20	10CL20CP
Diet 2	15	20	15CL20CP
Diet 3	20	20	20CL20CP
Diet 4	10	30	10CL30CP
Diet 5	15	30	15CL30CP
Diet 6	20	30	20CL30CP
Diet 7	10	40	10CL40CP
Diet 8	15	40	15CL40CP
Diet 9	20	40	20CL40CP

The proximate components analysed were as follows:

3.3.1.1 Moisture

Moisture was determined by drying samples in an advantec electric furnace maintained at 105^{o}C for 5 hours. The difference between the initial weight of the sample and that after drying was recorded and moisture calculated as follows:

$$\text{Moisture (\%)} = \frac{\text{Difference between before and after drying (g)}}{\text{Weight of sample before drying}} \text{ x } 100 \ldots\ldots 3.1$$

3.3.1.2 Crude protein (CP)

Crude protein levels were determined indirectly from the analysis of total nitrogen by the Micro-Kjeldahl method after acid digestion. The amount of protein in the sample was calculated by multiplying the amount of nitrogen by 6.25 (6.25 times the amount of total nitrogen in the sample that would equal the total amount or 100% of the protein in the sample). The percentage of crude protein was calculated as follows:

$$\text{Crude protein (\%)} = \frac{\text{Amount of protein in the sample (g)}}{\text{Weight of sample (g)}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

3.3.1.3 Ash

The ash was determined as total inorganic matter by incineration of the sample in an advantec electric furnace at 550^{o}C for 5 hours. The remaining inorganic material was reduced to their stable form, oxides or sulphates were considered as ash. Then the ash was calculated as follows:

$$\text{Ash (\%)} = \frac{\text{weight of ash}}{\text{weight of original sample}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

3.3.1.4 Crude fat

The crude fat was determined by extraction with petroleum ether for 16 hours in a Soxhlet apparatus. After drying the ether, the flasks containing the fat were dried in an oven for 8 hours at 85^{o}C. The ether was evaporated and the crude fat weighed. The ether extract was then calculated as follows:

$$\text{Crude fat (ether extract)}(\%) = \frac{\text{weight of crude fat}}{\text{weight of sample}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.4$$

3.3.1.5 Crude fibre

Crude fibre was determined by subjecting the residue from ether extraction to boiling in dilute sulfuric acid (1.25%) for 30 minutes, followed by boiling in dilute sodium hydroxide (1.25%) for another 30 minutes and then passing the solution through the filter. The material was then dried and weighed. The material was burnt in a furnace at 550^{o}C for 5 hours to oxidize off the crude fibre, and ash weight was obtained. The ash left was weighed and amount of crude fibre was calculated by subtracting the weight of ash from the weight of material left after boiling and drying. Then finally percentages were calculated as follows:

$$\text{Crude fibre (\%)} = \frac{\text{weight of crude fiber}}{\text{weight of original sample}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

3.3.1.6 Nitrogen – free extract (NFE)

This nitrogen – free extracts includes all the nutrients not assessed by proximate analysis. These are composed mainly of digestible carbohydrates, vitamins and other non-nitrogen soluble organic compounds. It is, therefore, calculated by subtracting the percentages calculated for each nutrient from 100 as illustrated below:

Nitrogen-free extract (%) = 100 - (A* + B + C + D + E)……3.6

Where:

A* = Moisture content (%), since the calculations were made on dry matter basis (with dehydrated material), it was excluded (FAO, 1994).

B = Crude protein content (%)

C = Crude lipid content (%)

D = Crude fibre content (%)

E = Ash content (%)

3.3.1.7 Gross energy determination

Gross energy was determined either directly calorimetrically or was calculated from protein, lipid and carbohydrates content.

3.3.1.7.1 Bomb calorimetry

Gross energy content of feed ingredients and fish samples were determined by igniting samples in an adiabatic bomb calorimeter (Nenken type, Parr – model 1013 – U, 1999). Total heat of combustion of the sample was determined by completely oxidizing the compound to carbon dioxide, water and other gases and measuring the heat released (AOAC, 2002).

3.3.1.7.2 Calculated gross energy

Gross energy was calculated using standard factors of 23.6, 39.5 and 17.2 kJ/g for protein, lipid and carbohydrates, respectively according to Jauncy (1998). Protein, lipid and carbohydrate energy contents were assumed to give total or gross sample energy.

3.4 Feed formulation procedures

The ingredients were ground and mixed thoroughly to achieve a homogenous sample. Water (5 – 10%) was then added before taken to a pellet making machine (BSW 330) attached with a 3.2mm metal die. Since one machine was used to make the pellets the first feed from the metal die after a turn was discarded to avoid contamination from the previous feed. The feed was then spread on the sacks and sundried for two days (Figure 3.4). Upon drying, the feed was put in the plastics bags and put in a cooler set at 8^{o}C till used.

Table 3.3: Composition of ingredients used to formulate the experimental diets

Ingredient	Moisture (%)	Total ash (%)	Crude fibre (%)	Crude fat (%)	Crude protein (%)	Carbohydrates (%)	Energy (Kcal/g)
Soya beans cake	6.8	5.2	4.1	10.6	46.6	26.7	3.89
Maize meal	10.2	2.6	11.1	7.9	9.1	59.1	3.44
SoyaGold Oil	-	-	0	100	0	0	8.84

Table 3.4: Composition of experimental diets (%) fed to *O. andersonii*

	Experimental diets								
	10CL20CP	**15CL20CP**	**20CL20CP**	**10CL30CP**	**15CL30CP**	**20CL30CP**	**10CL40CP**	**15CL40CP**	**20CL40CP**
Soya bean cake (%)	28.8	30.1	31.4	54.8	56.0	57.3	80.6	82.0	83.3
Maize bran (%)	67.8	61.2	54.7	42.6	36.0	29.5	17.4	10.8	4.3
SoyaGold oil (%)	1.4	6.7	12.0	0.6	5.9	11.2	0	5.2	10.4
[1]Vitamin premix (%)	1	1	1	1	1	1	1	1	1
[2]DCP (%)	1	1	1	1	1	1	1	1	1
Proximate analyses									
Moisture (%)	10.9	15.6	20.4	9.9	13.9	18.6	7.5	12.1	18.6
Crude protein (%)	20	20	20	30	30	30	40	40	40
Crude fat (%)	10	15	20	10	15	20	10	15	20
Crude ash (%)	3.3	3.2	3.1	4.0	3.9	3.8	4.7	4.6	3.8
Carbohydrates (%)	48.7	45.1	41.5	40.6	37.0	33.4	32.5	28.9	33.4
Crude fibre (%)	8.9	8.2	7.5	7.1	6.4	5.7	5.3	4.7	5.7
Gross energy (Kcal/g)	3.649	3.946	4.243	3.726	4.023	4.320	3.807	4.100	4.397
P/E (g protein/Kcal)	0.055	0.051	0.047	0.081	0.075	0.069	0.105	0.098	0.091

[1]Vitamin stress pack (100g): Retinol 2, 000, 000 *I.u*; Cholecalciferol 300, 000 *I.u;* Tocopherols 3000 *I. u;* Menadione 300mg; Ascorbic acid 3, 000mg; Riboflavin 500mg; Niacin 2, 500mg; Pantothenic acid 1, 000mg; Cobalamines 3mg; Pyridoxine 200mg; Folic acid 50mg and Thiamine 200mg.
[2]D-calcium phosphate

a) Feed production **b) Nine feeds being sun dried**

Figure 3.4: Feed production and drying

The total cost of the ingredients of the diets is given in Table 3.5.

Table 3.5: Production cost of the feed at the time of feed production

Feed code	Unit cost (ZK/Kg)
No feeding	0
10CL20CP	2, 397.38
15CL20CP	2, 421.29
20CL20CP	2, 445.20
10CL30CP	2, 991.27
15CL30CP	3, 015.18
20CL30CP	3, 039.10
10CL40CP	3, 583.68
15CL40CP	3, 609.08
20CL40CP	3, 632.98

ZK: Zambian Kwacha; exchange rate: US$1 = ZK5, 000 (2011)

3.5 Water quality parameters

Water temperature, pH and conductivity were monitored twice a week throughout the study period using a Horiba Hachit Kit U – 10 and the values of the water temperature (°C) and conductivity (ms/cm) read from the screen. Nitrite was determined twice a week analytically with samples read on a spectrophotometer HC 1000 model (appendix 10.1).

3.6 Data analysis

General Linear Model (univariate analysis procedure) was performed to determine the interactions and differences among protein and lipid levels which were deemed significant at $P< 0.05$ for parametric data. If interactions did not exist the combinations of the nutrient levels were treated as treatments and nutrients (lipid and protein) treated independently. Differences were separated by Duncan's Multiple Range Test (DMRT) (Duncan, 1955) for protein and lipid combinations while planned contrasts were used for independent nutrients (protein and lipid). To verify the trend of the lipid and protein on growt indices polynomial contrast was used. Before analysis, parametric data were tested for normality using Shapiro – Wilk test and the homogeneity of variance using Levene's test for Equality of Variances and appropriate transformation made with the help of gladder function in Stata 12.0 once these assumptions were violated.

Correlation analysis was performed to describe the strength and direction of treatments and growth, nutritional, reproductive characteristics and cost variables. If found strong ($r \leq -0.5$ or $r \geq 0.5$) and significant ($P < 0.05$) regression analysis was performed too.

Cross tabulations and, Chi-square (χ^2) and fisher's tests were used to examine the associations between the reproductive status of fish and nutrient (lipid and protein) combinations and the sex ratios according to MT treatment with the latter test used in an event of violating the assumptions for χ^2. Mann – Whitney U test was carried out to provide post – hoc comparisons of the Mean Ranks.

Statistical Package for Social Scientist (SPSS) 15.0 (SPSS Inc) and Stata 12.0 (StataCorp) softwares were used in analysing the data. Microsoft excel was used in the production of figures and graphs. Untransformed data are presented to facilitate interpretation.

CHAPTER 4

GROWTH, AND FEED UTILIZATION OF *OREOCHROMIS ANDERSONII* AND COST EFFECTIVENESS OF DIFFERENT COMBINATIONS OF SOYA BEAN BASED PROTEIN AND LIPID LEVELS

4.1 Introduction

Metabolisable energy taken in as feed which is not dissipated as heat is retained within the body as new tissue elements as growth, including any storage products or disseminated as gametes (Wootton, 1998). Therefore, growth and reproduction are complementary processes despite both of them relying on the limited resources. As the fish grows this energy is stored as protein and fat. The organismic growth is a change in length and weight or both with increasing age (Shammi and Bhatnagar, 2002). It can be calculated as changes in biomass during an interval of measurements and includes somatic (protein and lipid) growth and the development of gonads (Mateo, 2007). However, fish can change in length without changing in weight and vice – versa despite the two being correlated.

Growth is an important factor in fish farming since fast growth ensures large size, therefore, short culture period resulting into frequent harvests maximizing gross margins in a year. Weight gain is often a preferred trait to length increase since marketability is often on weight basis rather than volume. In addition, if fish is not being fed *ad libitum*,

feeding is based on the fish body mass. Growth also determines the suitability of a species for aquaculture under culture conditions. The rate of growth depends on the number of factors divided into exogenous factors imposed by the environment and endogenous factors related to the genotype and physiological condition of the fish. Of the environmental parameters that determine growth include temperature, dissolved oxygen (DO), salinity, photoperiod, degree of competition, age, state of maturity of fish and the type and the amount of the nutrient contained in the feed (Shammi and Bhatnagar, 2002). This is because the feed quality can differ both in the energy and nutrient content and size of the food particles (Wootton, 1998). Growth rate also depends on population density with higher densities showing slower growth and vice - versa.

Growth in fish is not constant throughout their life although it is indeterminate given the suitable environmental conditions but decreases with age. Young fish grow at a faster rate and this slows down as the fish advances in age. In addition, the difference in growth rate established by young fish does not persist throughout life. Initially slow – growing fishes may surpass initially fast growing fish and finally reach a greater length – at – age (Kinne, 1960; Pauley *et al*., 1996).

Protein is the main source of nitrogen and essential amino acids in animals. Tacon and Cowey (1985) showed that there is a linear relationship between daily protein requirement per unit body weight and specific growth rate. However, it is the most expensive source of energy in artificial feeds. Furthermore, excessive dietary protein may cause deterioration of water quality by increasing the ammonia excretion. However,

Yigit *et al*. (2003) found that the ammonia excretion rates might be affected by the source of protein and not the protein level within the protein quality. Therefore, it is necessary to keep the proportion of protein down to optimum levels necessary for good growth and feed conversion ratio (FCR) to reduce the cost and the nitrogen waste primarily ammonia. Dietary protein utilization can be improved by partially replacing dietary protein with lipid or carbohydrates to benefit from protein - sparing effect (De Silva *et al*., 1991; Du *et al*., 2009). Previously production of feed with highly contents of fat was difficult due to problems with extrusion. However, with the new improvements in extruder technology high fat content in dry feed is possible as such upper limit of fat content in salmonid feed has risen exceeding 30% (Guillaume *et al*., 2001). Lipids represent a concentrated cost effective energy source and therefore can be used in fish feeds either partially or completely to spare the protein. They are also the sources of essential fatty acids (EFAs).

In freshwater fishes, EFAs can usually be met by supplying the shorter – chain precursors: linolenic acid (18:3[n – 3]), linoleic acid (18:2[n – 6]) or although better growth performance can be often achieved by supplying the bioactive highly unsaturated fatty acids (HUFA) forms preformed in the diet such as eicosapentanoic acid (20:5[n – 6]), docosahexanoic (22:6[n – 3]) and arachidonic (20:4[n – 6]) (Kanazawa, 1985: Tidwel *et al*., 2007). However, excessive energy can reduce fish growth as a result of reduction in feed intake (reduction in protein intake) since fish eats to satisfy its maintenance requirement (Kefi, 2007) and reduction in the utilization of other nutrients (Takeda *et al.*, 1975; Shiau and Huang, 1990; Shiau and Lan, 1996). It can also result into increase in lipid deposition in the fish muscle. It is, therefore, important to obtain an optimal protein

to energy ratio for the most economical production of any fish enterprise (Shiau and Lan, 1996).

Fish farming in the sub Saharan countries was promoted mainly to reduce food insecurity and to improve nutrition in the rural poor country. However, this does not attach a commercial factor to the sector making it still dependent on developmental programmes It should be viewed as the means to generate income apart from improvement in nutrition and food diversification enterprise (Hishamunda, 2007). Feed accounts about up to 60% of the total operational cost of producing fish (Madalla, 2008; Virk and Saxena, 2003) and therefore high cost of the feed may derail the development of the sector. Availability of cost effective diet can make a difference between a profitable and unprofitable operation and may determine the economic viability of a fish farming operation (Madalla, 2008).

Oreochromis andersonii remains an important fish species in the Zambian aquaculture. In fact DOF has adopted it as the candidate indigenous fish species for fish farming. However, its dietary requirements remain unknown. The study was conducted to determine the optimum protein and lipid combinations from soya bean and maize the cheap plant based sources for growth and their effect on the whole body composition of *O. andersonii*. The cost effectiveness of these different combinations of the same diets was investigated too.

4.2 Materials and method

4.2.1 Pond and hapa preparations

The 750m^2 pond was drained completely and allowed to dry for two (2) days before mud was removed from the bottom. Soil samples from the pond were taken from the shallow, middle and upper parts for pH determination in the biological and chemical laboratory using nitrophenol method (Boyd, 1979). To reduce natural propagation of plankton, lime and fertilizers were not applied. The hapas, whose material was sourced from Japan and sown in 0.4m x 1.5m x 0.9m, were set in the 750m^2 semi – concrete pond. Bamboo sticks were cut and drilled in the pond to act as supporters for the hapas. To avoid fish escapes and predation by the birds, 5cm of each hapa was laid vertically into the soil and buried and the top of the hapas were covered with the white netting material. Water was then allowed to flow in the pond up to 20cm mark. The pond was again drained after two days. This was done to allow the frogs lay eggs and then make them stranded by draining water for them to die due to heat from direct sunlight. It was then allowed to dry again for two days before the pond was then filled with water again. After three (3) days the fish was then stocked from pond cages.

4.2.2 Experimental fish

The juvenile *O. andersonii* (Figure 4.1 and Table 4.1) were seined from the 2, 500m^2 fish pond and were put in the buckets for selection of similar sizes. A total of 500 fish were selected. The number of fish sampled for weighing and length measurement was based on the following formula:

$n = K(C/d)^2$……4.1

Where; n = Sample size;

K = Constant depending on the significance level (10%) and probability of detecting the difference (50%) = 5

C = Coefficient of variation (%) calculated as $(S/\bar{X})*100$; where S = standard deviation and $\bar{X}$ is the sample mean. From previous studies the CV (%) for *O. andersonii* is 37.22% (Kefi *et al.,* 2012)

d = Important difference between the group means to be detected (% of the mean) = 10%

$$n = 5 * \frac{37.22^2}{10^2}$$

$$n = 69$$

The fish were then taken and stocked in the pond cage (as described in section 3.2) for 22 days. In the pond cage the fish were fed twice a day at 5% body weight with the feed described in section 3.3.

Figure 4.1: Fingerlings in the seine net for experimental set up

Table 4.1: Weight and length of *O. andersonii* juveniles stocked in pond cage

Parameter	**Mean ± SD**
Weight (g)	4.03 ± 1.5
Standard length (mm)	44.8± 5.145
Total length (mm)	58.25± 6.544

4.2.3 Experimental design

The experiment started on 6th December 2010 through up to 2nd March 2011 (55 days). Forty (40) hapas fixed in a 750 ^{m2} semi – concrete pond (wall made of concrete with the bottom made of earth) were set in a Randomised Complete Block Design (RCBD) (Gomez and Gomez, 1984) factorial structure with three levels for lipid (10, 15 and 20%) and protein (20, 30 and 40%). The hapas were laid in a row (block) of 10 (0.5m between

and 2m within the row) across the pond since it had different water depths. Therefore, this allowed uniform water depth for each row (Figure 4.2).

Figure 4.2: Hapas ($0.6m^2$) being constructed for the experiment

For each treatment and unfed group there were four replicates. Each hapa was stocked with 10 fish totalling 40 fish for each treatment. There were no significant ($P > 0.05$) differences in mean weight (g), standard length (mm) and total length (mm) among the treatments (Table 4.2) of the fish stocked in all the 40 hapas.

Table 4.2: Weight and length of *O. andersonii* used in the experiment (mean ± SE)

Treatment	**Unfed**	**10CL20CP**	**15CL20CP**	**20CL20CP**	**10CL30CP**	**15CL30CP**	**20CL30CP**	**10CL40CP**	**15CL40CP**	**20CL40CP**
Weight (g)	5.185 ± 0.409	5.195 ± 0.411	5.333 ± 0.357	5.070 ± 0.349	6.110 ± 0.446	5.218 ± 0.357	5.390 ± 0.354	5.500 ± 0.354	6.328 ± 0.449	6.290 ± 0.363
SL (mm)	47.650 ± 1.213	47.625 ± 1.078	48.575 ± 1.035	47.500 ± 1.077	48.675 ± 1.529	47.450 ± 1.093	48.375 ± 1.056	49.000 ± 1.049	50.950 ± 1.450	51.075 ± 0.993
TL (mm)	63.400 ± 1.652	63.450 ± 1.543	64.925 ± 1.311	64.175 ± 1.579	67.050 ± 1.464	63.625 ± 1.463	64.500 ± 1.381	65.900 ± 1.394	68.100 ± 1.811	67.875 ± 1.293

4.2.4 Fish stocking and sampling

The pond cage was lifted and drifted to the dyke before a scoop net was used to catch the fish. Fish were immediately put in the buckets and taken to the pond prepared with hapas. The fish were then selected randomly for individual weighing and length measurements according to the procedures described by Skelton (2001). Fish were anaesthetized in crude clove powder (5g/L) to reduce stress before they were put on the weighing balance and measuring board for weight and length (SL and TL) determination, respectively. In addition, forty five fish (45) were also sampled randomly, killed and frozen for proximate analyses as described in section 3.3.1. Samples were analysed for moisture, crude protein, crude lipid, NFE, gross energy and ash and results expressed as percentage of the live weight. Mortality was monitored daily and fish that died was not replaced. At the end of the experiment the fish were weighed and length (SL and TL) taken. The fish were then taken to the biological and chemical laboratory for the extraction of the liver. The carcasses were then frozen till proximate analyses conducted as described in section 3.3.1.

4.2.5 Feeding regime

Fish were hand fed twice a day (10:00 hours and 15:00 hours) at 5% body weight using the experimental diet formulated as described in section 3.2. The feed allowance was adjusted only when mortality was observed in the hapa.

4.2.6 Water quality parameters

Selected water quality parameters were collected as described in section 3.5

4.3 Statistical analysis

Several growth, organ and feed utilization performance parameters were calculated with the following equations;

$$\text{Body Weight gain (BWG) (g)} = \text{Mf (g)} - \text{Mi (g)} \quad \ldots 4.2$$

$$\text{Specific growth rate (SGR)(\%)} = \frac{\text{Lnf(g)} - \text{Lni(g)}}{\text{t}} \times 100 \quad \ldots 4.3$$

$$\text{Apparent feed conversion effeciency (AFCE)(\%)} = \frac{\text{Weight gain (g)}}{\text{Total feed provided (g)}} \times 100 \quad \ldots 4.4$$

$$\text{Apparent Protein efficiency ratios (APERs)} = \frac{\text{Mf (g)} - \text{Mi (g)}}{\text{total protein provided (g)}} \quad \ldots 4.5$$

$$\text{Apparent Percent protein deposited (PPD)} = \frac{\text{Pf (g)} - \text{Pi(g)}}{\text{total protein provided}} \times 100 \quad \ldots 4.6$$

$$\text{Apparent Percent lipid deposited (PLD)(\%)} = \frac{\text{Lf (g)} - \text{Li(g)}}{\text{total lipid provided}} \times 100 \quad \ldots 4.7$$

$$\text{Condition factor (k)} = \frac{\text{Weight of fish (g)}}{\text{SL(mm)}^3} \times 100{,}000 \quad \ldots 4.8$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fish at the end of the experiment}}{\text{Number of fish at the start of the experiment}} \times 100 \quad \ldots 4.9$$

Where Mf is the final mean weight (FMW); Mi is the initial mean weight; f is the final weight; i is the initial weight; Lnf is the natural logarithm of final weight; Lni is the natural logarithm of the initial fish weight; Pf is final body protein (g); Pi is the initial body protein (g); Lf is the final body lipid (g); Li is the initial body lipid (g); t is the

experimental period (55 days). Data collected and calculated on the growth, organ and feed utilization performance parameters were analysed as described in section 3.7.

4.3.1 Cost analysis

Gross margin analysis was performed to determine the cost effectiveness of the prepared diets. It was assumed that all other operating costs remained constant and only the variable cost of ingredients was used in calculations. The cost of the diets was calculated using the prevailing prices for the feed ingredients in Zambia at the time of the experiment as follows; soya bean ZK2, 700/kg, maize bran ZK600/kg, SoyaGold oil ZK5, 600/L, vitamin stress pack ZK90, 000/kg and DCP ZK14, 000/kg. The final fish weight was assumed to be the harvest weight of fish. The following key economic indicators were computed according to Jolly and Clonts (1993).

$Y = P_sS + P_mM + P_oO + P_vV + P_dD$ ----------4.10

Where Y = Total Cost (TC) of feed for a given prepared diet

P_s = Unit cost of Soya bean S.

P_m = Unit cost of maize bran M.

P_o = Unit cost of soyaGold oil O.

P_v = Unit cost of vitamin V.

P_d = Unit cost of DCP D.

$TR = P*FW$----------4.11

Where; TR = Total revenue; FW final fish weights and P the unit price.

$\prod = TR - Y$ ----------4.12

Where; $\prod$ is gross margin, Y and TR as described in equations i and ii above

Multiple regression was performed in order to identify parameters that correlated and affected the gross margin. The model used is as follows:

$\prod = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + \mathcal{E}$----------4.13

Where:

$\prod$ = Dependent variable (Gross margin)

X_1 to X_6 = Independent variables

X_1 = Protein level

X_2 = Lipid level

X_3 = Body weight gain

X_4 = SGR

X_5 = APER

X_6 = interaction effect between protein and lipid if existed

b_1 to b_6 = regression coefficients

a = Constant

$\mathcal{E}$ = Residual

4.4 Results

4.4.1 Protein and lipid combinations on growth performance and feed utilisation

4.4.1.1 Interaction effect of protein and lipid on *O. andersonii*

The interaction effect of protein and lipid on final body weight (g), body weight gain (g), SGR (% day^{-1}), *K*, AFCE (%), PERs and GM (ZK) was not significant ($P > 0.05$) (Table 4.3).

Table 4.3: Significant levels for the interaction effect between protein and lipid levels

Parameter	**F – value**	***P* – value**
Final weight (g)	2.253	0.064
Body weight gain (g)	0.961	0.429
SGR (% day^{-1})	0.059	0.993
K	0.618	0.650
AFCE (%)	0.080	0.987
APERs	0.496	0.739
GM (ZK)	0.345	0.844

A two way analysis of variance yielded a significant interaction ($P < 0.05$) between the lipid and protein levels on PPD and PLD. This suggests that the effect of lipid depended on protein on the deposition of both the lipid and protein. An analysis on lipid levels indicated significant differences ($P < 0.05$) among the three levels. Duncan's Multiple Range Test showed that at 10% lipid level the means of PPD for 30% and 40% protein

were similar ($P > 0.05$). At 15% lipid level, the PPD was significantly higher ($P < 0.05$) at 30% crude protein. Although no significant differences ($P > 0.05$) were observed at 20% lipid level between 20% and 30% protein the PPD was highest at latter protein level (Table 4.4).

Table 4.4: Per cent protein deposition in *O. andersonii* fed different levels of protein

	Protein level (%)		
Lipid level (%)	**20**	**30**	**40**
10	252.251 ± 25.172^{b}	201.642 ± 15.462^{a}	179.106 ± 12.229^{a}
15	258.208 ± 22.353^{a}	324.798 ± 22.640^{b}	211.328 ± 15.171^{a}
20	269.741 ± 16.362^{b}	287.646 ± 24.640^{b}	208.542 ± 16.975^{a}

Different superscripts in a row indicate significant difference ($P < 0.05$).

Similarly the univariate analysis showed a significant interaction ($P < 0.05$) between the lipid and protein levels on per cent lipid deposition. Splitting data according to lipid levels significant differences ($P < 0.05$) were observed at all levels. The highest PLD was observed at 20% crude protein and this was significant ($P < 0.05$) across the other two levels of protein. At 15% and 20% lipid levels, there was a reduction on the PLD. At 15% lipid level, the lowest reduction was achieved at the highest level of protein while at 20% crude protein the reduction of PLD was significantly highest. At 20% lipid level the increase in PLD was only observed at 30% crude protein (Table 4.5).

Table 4.5: Per cent lipid deposition in *O. andersonii* fed different levels of protein

	Protein level (%)		
Lipid level (%)	**20**	**30**	**40**
10	497.295±35.537^{c}	-43.555±3.340^{a}	175.125±11.957^{b}
15	-334.152±28.927^{a}	-223.517±15.714^{b}	-209.870±15.067^{b}
20	-217.291±13.181^{a}	73.732±6.316^{c}	-48.218±3.925^{b}

Different superscripts in a row indicate significant difference ($P < 0.05$).

The fish fed that the prepared diets had a significant ($P< 0.05$) higher final weight than that of the unfed group although significant differences ($P < 0.05$) existed within the fed group despite the initial weight being insignificant ($P> 0.05$) (Table 4.6).

There were significant differences ($P< 0.05$) among the treatments in the BWG (g). The highest BWG (25.668 ± 1.332g) was recorded in the fish fed with the diet (20CL40CP) although this was not significant different ($P> 0.05$) from the fish fed with feeds incorporated with protein sources at 30% and 40%. Among the fed group the lowest BWG (g) was observed in fish fed with 20CL20CP (18.028 ± 1.233g) although this was not significant different ($P> 0.05$) from the fish fed the other 20% protein incorporated diets.

Similarly, significant differences ($P < 0.05$) existed among the treatments in the SGR (%day^{-1}). The diet 20CL40CP had the highest SGR (%day^{-1}) (3.297 ± 0.170%) although this was not significant different ($P> 0.05$) from the 15CL20CP feed and the feed

combined with the 30% and 40% protein level. The SGRs (%day^{-1}) for the feed 10CL20CP and that of 20CL20CP feed were not significant different (P> 0.05) from each other. The lowest SGR (%day^{-1}) was observed in the unfed group in the 55 days experimental period (Table 4.6).

There were no significant differences (P> 0.05) among the treatments in the K among the treatments. However, the fish reared on the diet 15CL40CP (2.544 ± 0.144) recorded the highest K followed by the unfed group (2.515 ± 0.180). The least K was recorded in the fish fed with 20CL40CP (2.202 ± 0.092) diet (Table 4.6).

Significant differences (P< 0.05) existed in the APERs. The fish fed on 10CL20CP (11.505 ± 0.967) had the highest APER although this was not significantly different (P> 0.05) from the fish raised on other 20% and 30% protein level. The lowest APER was observed in fish fed with the 20CL40CP feed (Table 4.6).

Apparent feed conversion efficiency was highest on the feed 15CL40CP (294.119±27.016%) and this was significantly different (P< 0.05) from the feed 20CL20CP but not different (P> 0.05) from the rest of the feeds (Table 4.6).

Table 4.6: Growth performance and nutrient utilisation of *O. andersonii* fed with nutrient combinations (mean ± SE)

Treatment	Unfed	10CL20CP	15CL20CP	20CL20CP	10CL30CP	15CL30CP	20CL30CP	10CL40CP	15CL40CP	20CL40CP
Final weight (g)	15.596 ± 1.040^{a}	23.548 ± 1.03^{b}	26.535 ± 1.380^{b}	23.338 ± 1.400^{b}	30.068 ± 1.214^{cd}	28.593 ± 1.276^{c}	29.900 ± 1.293^{cd}	30.017 ± 1.078^{cd}	28.737 ± 1.499^{cd}	32.771 ± 1.679^{d}
SGR (% day^{-1})	2.140 ± 0.167^{a}	2.969 ± 0.170^{bc}	2.890 ± 0.167^{bc}	2.662 ± 0.154^{b}	3.078 ± 0.183^{bc}	3.126 ± 0.167^{bc}	3.267 ± 0.167^{c}	3.232 ± 0.167^{c}	3.297 ± 0.170^{c}	3.176 ± 0.167^{bc}
BWG (g)	11.971 ± 1.332^{a}	18.628 ± 1.355^{b}	20.687 ± 1.332^{bc}	18.028 ± 1.233^{b}	24.200 ± 1.459^{cd}	23.059 ± 1.332^{cd}	24.040 ± 1.332^{cd}	24.411 ± 1.332^{cd}	23.653 ± 1.653^{cd}	25.668 ± 1.332^{d}
K	2.515 ± 0.18^{a}	2.437 ± 0.105^{a}	2.267 ± 0.086^{a}	2.202 ± 0.092^{a}	2.449 ± 0.088^{a}	2.312 ± 0.074^{a}	2.241 ± 0.070^{a}	2.429 ± 0.115^{a}	2.544 ± 0.144^{a}	2.236 ± 0.10^{a}
APERs	-	11.505 ± 0.967^{c}	11.254 ± 0.951^{bc}	9.141 ± 0.880^{abc}	8.198 ± 1.042^{abc}	8.243 ± 0.951^{abc}	8.963 ± 0.951^{abc}	8.963 ± 0.951^{a}	6.852 ± 0.951^{ab}	7.353 ± 0.967^{a}
AFCE (%)	-	230.106 ±27.016^{ab}	225.087 ±26.561^{ab}	182.817±24.591^{a}	245.932±29.097^{ab}	247.304±26.561^{ab}	268.882±26.561^{b}	274.082±26.561^{b}	294.119±27.016^{b}	256.781±26.561^{ab}
Survival rate (%)*	96.67	100.00	100.00	96.67	100.00	100.00	100.00	96.67	100.00	100.00

Different superscripts in a row indicate significant difference ($P < 0.05$). *No statistical analysis was possible as determinations were performed on pooled samples.

Body composition data for whole fish carcasses are shown in Table 4.7. The body crude protein was not significant ($P > 0.05$) with the highest being recorded with the fish fed 15CL30CP feed (57.300 ± 0.577%). However, body protein for fish before the initiation of the experiment was lower than the fish at the end of the experiment. No significant differences ($P > 0.05$) were observed in the crude lipid of the body composition of the fish subjected to the treatments. The highest body total lipid was observed in fish carcass fed with 10CL20CP (21.950 ± 0.779%). The lowest lipid level in fish carcass was observed in 15CL30CP (12.800 ± 3.406%).

There were no significant differences ($P > 0.05$) observed in the all water quality parameters (water temperature, nitrite, pH, conductivity and DO) determined among the treatments (Table 4.8).

Table 4.7: Body composition of *O. andersonii* fed different combinations of nutrients (mean ± SE)

Parameter	Initial	Unfed	10CL20CP	15CL20CP	20CL20CP	10CL30CP	15CL30CP	20CL30CP	10CL40CP	15CL40CP	20CL40CP
Moisture (%)	9.286± 0.196^{a}	18.750 ± 7.887^{a}	45.762 ± 2.335^{bc}	53.760 ± 0.721^{e}	50.203 ± 0.900^{cde}	50.797 ± 1.602^{de}	45.616 ± 0.780^{b}	46.134 ± 1.945^{bc}	50.738 ± 0.818^{de}	51.271 ± 1.319^{de}	48.558 ± 0.397^{bcd}
Ash (%)	5.538 ± 0.528^{a}	3.464 ± 0.181^{a}	4.979 ± 1.115^{a}	15.343 ± 1.360^{b}	16.822 ± 2.209^{b}	15.290 ± 0.274^{b}	15.864 ± 1.094^{b}	14.677 ± 1.924^{b}	14.493 ± 0.692^{b}	14.497 ± 0.973^{b}	16.624 ± 0.282^{b}
Crude protein (%)	48.000 ± 0.866^{a}	56.000 ± 0.058^{b}	53.950 ± 0.375^{b}	53.100 ± 0.750^{b}	53.400 ± 0.173^{b}	54.250 ± 1.530^{b}	57.300 ± 0.577^{b}	55.900 ± 2.425^{b}	54.750 ± 3.088^{b}	55.250 ± 3.147^{b}	56.650 ± 2.800^{b}
Crude lipid (%)	17.750 ± 3.377	15.850 ± 2.569	21.950 ± 0.779	12.800 ± 3.406	13.400 ± 1.097	17.300 ± 0.635	14.550 ± 3.147	19.100 ± 4.215	19.400 ± 4.272	15.050 ± 0.491	16.750 ± 3.262^{a}
Crude fibre (%)	11.520 ± 0.082^{b}	10.170 ± 0.470^{a}	11.370 ± 0.370^{b}	11.220 ± 0.168^{b}	11.036 ± 0.036^{b}	11.386 ± 0.176^{b}	11.103 ± 0.116^{b}	11.070 ± 0.035^{b}	11.103 ± 0.003^{b}	11.003 ± 0.060^{b}	10.820 ± 0.151^{ab}
NFE (%)	17.192 ± 3.431^{c}	14.517 ± 3.032^{bc}	7.751 ± 1.161^{b}	7.537 ± 3.082^{ab}	5.342 ± 1.503^{a}	1.773 ± 0.864^{a}	1.183 ± 3.560^{a}	0.747 ± 3.330^{a}	0.253 ± 1.425^{a}	4.199 ± 2.281^{a}	0.844 ± 0.553^{a}
Gross energy (kJ/g)	21.296± 0.949^{bcd}	21.974 ±0.507^{cd}	22.736 ± 0.390^{d}	18.884 ± 0.708^{a}	18.814 ± 0.602^{a}	19.942 ± 0.056^{ab}	19.474 ± 0.497^{ab}	20.608 ± 0.649^{abc}	20.628 ± 0.754^{abc}	19.706 ± 0.226^{ab}	19.841 ± 0.536^{ab}

Different superscripts in a row indicate significant difference ($P < 0.05$).

Table 4.8: Water quality parameters

Treatment	**Unfed**	**10CL20CP**	**15CL20CP**	**20CL20CP**	**10CL30CP**	**15CL30CP**	**20CL30CP**	**10CL40CP**	**15CL40CP**	**20CL40CP**
Water Temperature (°C)	23.678±0.132	23.677±0.132	23.684±0.131	23.677±0.132	23.677±0.132	23.676±0.133	23.676±0.133	23.679±0.132	23.675±0.132	23.678±0.132
pH	7.77±0.02	7.77±0.06	7.77±0.06	7.76±0.06	7.77±0.06	7.77±0.06	7.76±0.06	7.77±0.06	7.77±0.06	7.77±0.06
Conductivity(µmho/cm)	0.776±0.082	0.762±0.080	0.754±0.088	0.758±0.082	0.760±0.073	0.750±0.086	0.764±0.077	0.758±0.080	0.754±0.082	0.772±0.077
Nitrite (NO_2^-N) (mg/L)	0.169±0.074	0.122±0.048	0.157±0.058	0.158±0.061	0.155±0.031	0.094±0.060	0.144±0.054	0.201±0.060	0.138±0.042	0.162±0.055

4.4.2 Optimal protein level on the growth of *O. andersonii*

Significant differences ($P< 0.05$) existed among the treatments. Although the BWG (g) for fish fed with 40% protein level (30.411 ± 0.824g) was higher than that of fish that fed 30% level (29.501 ± 0.753) the difference was not significant ($P> 0.05$) (Figure 4.3).

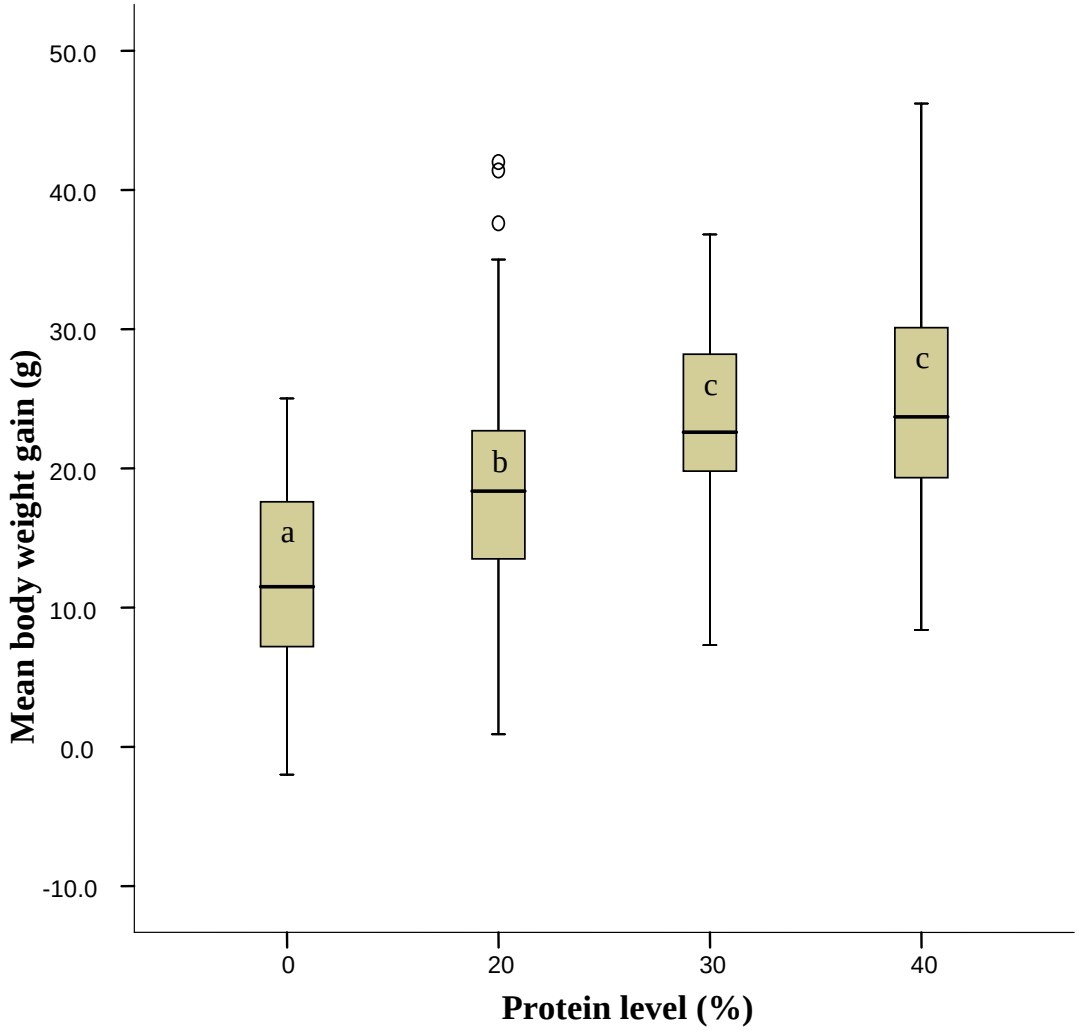


Figure 4.3: Box plot showing the mean body weight gain of fish (g) according to the crude protein level (%) (Different letters in the box plot are significant ($P< 0.05$))

Polynomial regression analysis showed a positive linear relationship (Y = 0.318x + 12.797, F = 4773, r = 0.97) between the mean BWG (g) and the protein level with an increase in BWG associated with an increase in protein level (Figure 4.4).

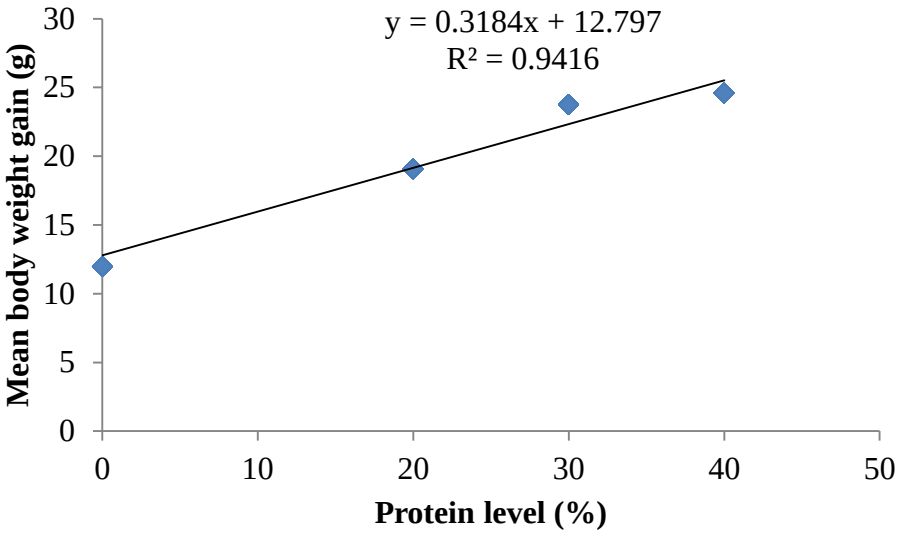


Figure 4.4: Effect of dietary protein level on BWG (g) of *O. andersonii*

There were significant differences ($P< 0.0\%$) in the SGR (% day^{-1}) of *O. andersonii* among the fed group. Although the 30% protein level fed fish was not significantly different ($P> 0.05$) from the 40% protein level fed fish the latter recorded the highest SGR (% day^{-1}) (3.235 ± 0.097%). The unfed group showed the lowest SGR (% day^{-1}) (2.140±0.167%) and this was significantly ($P< 0.05$) lower than the fed fish (Table 4.9).

Significant differences ($P< 0.05$) existed in the APERs across the treatments and these declined with an increase in the protein level (r = -0.282, n = 268, $P< 0.05$). The APER for fish fed with the least protein level (10.633 ± 0.539) exhibited the highest APER and

this was significantly ($P< 0.05$) higher than the fish given the 30% protein level (8.468 ± 0.567). The 40% crude protein level had significantly lowest ($P< 0.05$) APER (6.552 ± 0.551). The AFCE (%) means were significant ($P< 0.05$) among all the treatments with the highest being recorded in fish fed with the highest protein level although this was not significant different from the 30% crude protein (Table 4.9).

Table 4.9: Growth performance of the *O. andersonii* at different levels of protein

Treatment	0%	20%	30%	40%
FMW (g)	15.596 ± 1.040^{a}	24.436 ± 0.735^{b}	29.501 ± 0.753^{c}	30.411 ± 0.824^{c}
BWG (g)	11.971 ± 1.332^{a}	19.114 ± 0.755^{b}	23.766 ± 0.794^{c}	24.577 ± 0.773^{c}
SGR (% day^{-1})	2.140 ± 0.167^{a}	2.840 ± 0.095^{b}	3.157 ± 0.100^{c}	3.235 ± 0.097^{c}
K	2.515±0.181	2.299±0.055	2.333±0.045	2.410±0.071
APERs	-	10.633 ± 0.539^{c}	8.468 ± 0.567^{b}	6.552 ± 0.551^{a}
AFCE (%)	-	210.897 ± 14.942^{a}	254.514 ± 15.713^{ab}	274.779 ± 15.356^{b}
Survival rate (%)	96.67	96.67	100.00	96.67

Different superscripts in a row indicate significant difference ($P < 0.05$).

The carcass crude protein of the initial fish sample was significantly lower ($P< 0.05$) than the fish carcass at the end of the experiment in all the treatments. No significant differences ($P> 0.05$) were observed in all treatments at the end of the experiment. The carcass lipid was similar in all the treatments and was not significant different ($P> 0.05$) from the fish carcass at the start of the experiment (Table 4.10).

There were no significant differences ($P > 0.05$) in the selected water quality parameters taken (Table 4.11).

Table 4.10: Proximate composition of the *O. andersonii* at different levels of protein (mean ± SE)

Proximate composition	Initial	0%	20%	30%	40%
Moisture (%)	9.286 ±0.196[a]	18.750±7.887[a]	49.908 ± 1.380[b]	47.516 ± 1.122[b]	50.189 ± 0.621[b]
Ash (%)	5.538±0.528[a]	3.464 ± 0.181[a]	12.382 ± 2.033[b]	15.277 ± 0.666[b]	15.205 ± 0.501[b]
Crude protein (%)	48.000±0.866[a]	56.000± 0.0577[b]	53.483 ± 0.277[b]	55.817 ± 0.952[b]	55.550 ± 1.534[b]
Crude lipid (%)	17.750±3.377	15.850 ± 2.569	16.050 ± 1.817	16.983 ± 1.666	17.067 ± 1.682
Crude fibre (%)	11.520±0.298[c]	10.170 ± 0.470[a]	11.209 ± 0.127[cd]	11.186 ± 0.079[cd]	10.975 ± 0.063[b]
NFE (%)	17.192±3.430[c]	14.517 ± 3.032[c]	6.877 ± 1.114[b]	0.737 ± 1.479[a]	1.203 ± 1.102[a]
Gross energy (kJ/g)	21.296 ± 0.949	21.974 ± 0.507	20.145 ± 0.710	20.008 ± 0.288	20.058 ± 0.310

Different superscripts in a row indicate significant difference ($P < 0.05$).

Table 4.11: Water quality parameters of protein experiment (mean ± SE)

Treatment	**0%**	**20%**	**30%**	**40%**
Temperature (°C)	23.678 ± 0.132	23.679 ± 0.076	23.679 ± 0.076	23.679 ± 0.076
pH	7.78 ± 0.06	7.76 ± 0.04	7.77 ± 0.03	7.77 ± 0.03
Conductivity (µmho/cm)	0.776 ±0.082	0.758 ± 0.044	0.758 ± 0.042	0.76 ± 0.043
Nitrite (NO_2^-) (mg/L)	0.169±0.74	0.145±0.029	0.131 ± 0.027	0.167 ± 0.028

Different superscripts in a row indicate significant difference ($P < 0.05$).

4.4.3 Optimal lipid level on the growth of *O. andersonii*

There were significant differences ($P< 0.05$) in the final weights of fish between the unfed group and the fish subjected to the treatments. However, the final weights of the fish within the treatments were insignificant ($P> 0.05$) although the 20% lipid level (28.179 ± 0.827g) produced fish with the highest weight followed by 15% (27.920 ± 0.822g) and 10% (27.772 ± 0.822g) lipid level in that order. The trend was similar with mean fish body weight gain with the highest lipid level (20%) producing the fish with the highest weight gain (Table 4.12).

Similarly, there were significant differences ($P< 0.05$) in SGR (%day^{-1}) between the fed fish and unfed fish. No significant differences ($P> 0.05$) were observed in the fed fish subjected to the treatments. However, SGR (%day^{-1}) for fish fed at 15% lipid level (3.111± 0.102% day^{-1}) was the highest followed by the 10% lipid level (3.097 ± 0.102%

day^{-1}) with fish fed at 20% lipid level (3.022 ± 0.103% day^{-1}) exhibiting the lowest SGR(%day^{-1}) (Table 4.12).

The AFCE (%) was not significant (P> 0.05) among the lipid levels. However, the 15% lipid level (260.869 ± 167.272%) gave the highest AFCE followed by 10% lipid level (251.543 ± 135.774%). The fish subjected to 20% lipid level (241.072 ± 147.205%) gave the lowest AFCE (%). Similar trend was observed in APER with the highest lipid level having the lowest protein utilization (Table 4.12).

Proximate composition of *O. andersonii* used in the experiment is presented in Table 4.13. With exception of crude lipid the other parameters were significant (P< 0.05) across the treatments. The initial fish body crude protein composition was significantly (P< 0.05) lower that the crude protein in the fish at the end of the experiment (P> 0.05). The body crude lipid was not significant (P> 0.05) among the treatments (Table 4.13).

There were no significant differences (P> 0.05) in the selected water quality parameters determined (Table 4.14).

Table 4.12: Growth performance and feed utilization of soya bean based lipid by *O. andersonii* (mean ± SE)

Treatment	0%	10%	15%	20%
Final weight (g)	15.596 ± 1.040^{a}	27.772 ± 0.716^{b}	27.921 ± 0.800^{b}	28.179 ± 0.944^{b}
SGR (% day^{-1})	1.973 ± 0.192^{a}	3.097 ± 0.102^{b}	3.111 ± 0.102^{b}	3.022 ± 0.103^{b}
BWG (g)	9.976 ± 1.561^{a}	22.371 ± 0.857^{b}	22.573 ± 0.857^{b}	22.645 ± 0.862^{b}
K	2.515 ± 0.114^{b}	2.438 ± 0.060^{ab}	2.372 ± 0.061^{ab}	2.224 ± 0.051^{a}
APER	-	8.916 ± 0.606	9.212 ± 0.606	8.555 ± 0.609
AFCE (%)	-	251.543 ± 135.774	260.869 ± 167.272	241.072 ± 147.205
*Survival rate (%)	96.67	96.67	100	96.67

Different superscripts in a row indicate significant difference ($P < 0.05$). *No statistical analysis was possible as determinations were performed on pooled samples

Table 4.13: Proximate composition of the *O. andersonii* at different levels of lipid (mean ± SE)

Treatment	Initial	0%	10%	15%	20%
Moisture (%)	9.286 ± 0.196^{a}	18.750 ± 7.887^{a}	49.099 ± 1.192^{b}	50.216 ± 1.300^{b}	48.298 ± 0.863^{b}
Ash (%)	5.538 ± 0.528^{a}	3.464 ± 0.181^{a}	11.588 ± 1.701^{b}	15.235 ± 0.610^{bc}	16.041 ± 0.916^{c}
Crude protein (%)	48.000 ± 0.866^{a}	56.000 ± 0.058^{b}	54.317 ± 1.001^{b}	55.217 ± 1.126^{b}	55.317 ± 1.178^{b}
Crude lipid (%)	17.750 ± 3.377	15.850 ± 2.569	19.550 ± 1.434	14.133 ± 1.389	16.417 ± 1.775
Crude fibre (%)	11.520 ± 0.298^{c}	10.170 ± 0.470^{a}	11.286 ± 0.126^{bc}	11.109 ± 0.069^{bc}	10.975 ± 0.060^{b}
NFE (%)	17.192 ± 3.431^{b}	14.517 ± 3.032^{b}	3.259 ± 1.286^{a}	4.307 ± 1.767^{a}	1.250 ± 1.478^{a}
Gross energy (kJ/g)	21.300 ± 0.949^{bc}	21.974 ± 0.507^{c}	21.102 ± 0.487^{bc}	19.355 ± 0.286^{a}	19.754 ± 0.396^{ab}

Different superscripts in a row indicate significant difference ($P < 0.05$)

Table 4.14: Water quality parameters for different lipid levels (mean ± SE)

Treatment	0%	10%	15%	20%
Temperature (°C)	23.678 ± 0.132	23.677 ± 0.076	23.682 ± 0.079	23.676 ± 0.076
pH	7.78 ± 0.06	7.77 ± 0.03	7.77 ± 0.03	7.77 ± 0.03
Conductivity (µmho/cm)	0.776 ±0.082	0.760 ±0.042	0.753±0.045	0.765 ±0.042
Nitrite (NO_2^-) (mg/L)	0.169±0.74	0.159±0.074	0.129±0.028	0.155±0.028

A polynomial Regression analysis showed that a second order polynomial would describe the relationship ($Y = -0.0466x^2 + 1.4255x + 12.221$; $F = 5079$; $r = 0.988$) between the lipid level and final mean fish weight gain (g). A differential equation shows that maximum mean weight gain would occur at lipid level of approximately 15.3% (Figure 4.5).

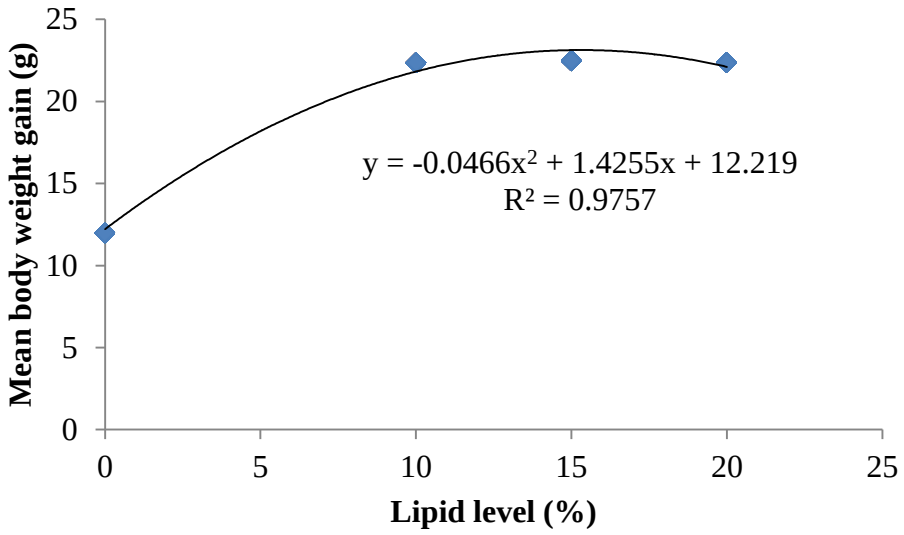


Figure 4.5: Relationship between the lipid level and mean fish body weight gain (g)

4.4.4 Cost analysis of the experimental diets

The cost of the protein source increased with the protein level inclusion and this was significant ($P< 0.05$) among the treatments. The cost of the 20CL40CP feed (ZK46.17 ± 2.95) was the most expensive and this was significant different ($P< 0.05$) from the rest of the experimental diets. This was followed by the feed 10CL40CP (ZK37.30 ± 2.68) although this was not significant different ($P> 0.05$) from the 15CL40CP, 20CL30CP,

15CL30CP, 10CL30CP, 20CL20CP, 15CL20CP feeds. The lowest cost was achieved at the 10CL20CP combinations although this was not significant different (P> 0.05) from the 15CL20CP feed. In terms of individual nutritional components, the cost of protein increased with the level of protein inclusion although the cost of 30% and 40% protein were not significant different (P> 0.05) from each other but significantly higher than the 20% protein level. A similar trend was observed in the lipid although the cost of the lipid at all levels was not significant (P> 0.05) (Table 4.15).

The 20CL40CP had the highest TR (ZK393.25 ± 16.68) although this was not significant different (P> 0.05) from the 10CL40CP (ZK360.21 ± 15.17), 20CL30CP (ZK358.80 ± 16.02), 15CL30CP (ZK343.11 ± 15.72) and 10CL30CP (ZK 360.82 ± 16.32). Total revenue for the protein level was highest at 40% level although this was not significant different (P> 0.05) from the 30% level. The unfed group had significantly lower (P< 0.05) TR than the 20% crude protein. In terms of lipid level, the unfed group had the lowest TR although no significant differences (P > 0.05) were observed among the fed group (Table 4.16).

Significant differences (P< 0.05) were observed in the GM among the combinations of protein and lipid. The highest was found in fish fed with 20CL40CP combination. However, this was not significant different (P> 0.05) from all combinations with 30% and 40% protein level. The unfed group produced the lowest GM. The GMs for 30% and 40% protein were not significant different (P> 0.05) from each other although the latter was higher than the former protein level. Although the 20% protein level was

significantly lower ($P< 0.05$) than the 30% and 40%, it was significantly higher ($P< 0.05$) than the unfed group. In terms of the lipid level, no significant differences ($P > 0.05$) among the treatments were observed. However, the unfed group had a significant lower ($P< 0.05$) GM than the fed group (Table 4.17).

Multiple regression results are presented in Table 4.18. Body weight gain (g), SGR (%day^{-1}) AFCE (%) and lipid level contributed significantly to the model with SGR (%day^{-1}) having the highest contribution followed by BWG (g).

Table 4.15: Cost analysis of protein level in the diet fed to *O. andersonii* (mean ± SE)

Treatment	**0%**	**20%**	**30%**	**40%**
Cost of soya bean (ZK)	0	9.12 ± 0.76^{a}	17.23 ± 0.80^{b}	24.78 ± 0.78^{c}
Cost of maize bran (ZK)	0	4.12 ± 0.12^{c}	2.48 ± 0.13^{b}	0.72 ± 0.12^{a}
Cost of soyaGold oil (ZK)	0	4.34 ± 0.20^{b}	3.68 ± 0.21^{a}	3.34 ± 0.20^{a}
Cost of Vitamin (ZK)	0	10.08 ± 0.43	10.26 ± 0.45	10.07 ± 0.44
Cost of DCP (ZK)	0	1.57 ± 0.07	1.60 ± 0.07	1.57 ± 0.07
FW (g)	19.727 ± 4.496^{a}	25.772 ± 1.459^{ab}	29.076 ± 1.28^{b}	28.831 ± 2.414^{b}
TC (ZK)	0	29.50 ± 2.67^{a}	35.32 ± 1.51^{b}	40.49 ± 1.55^{b}
TR (ZK)	187.15 ± 16.44^{a}	293.23 ± 8.67^{b}	354.02 ± 9.31^{c}	364.94 ± 9.19^{c}
∏ (ZK)	187.15 ± 16.44^{a}	264.01 ± 8.82^{b}	319.39 ± 9.47^{c}	325.31 ± 9.35^{c}

Different superscripts in a row indicate significant difference ($P < 0.05$).

Table 4.16: Cost analysis of lipid level in the diet fed to *O. andersonii* (mean ± SE)

Treatment	**0%**	**10%**	**15%**	**20%**
Cost of soya bean (ZK)	0	16.73 ± 0.81	16.28 ± 0.78	18.01 ± 76
Cost of maize bran (ZK)	0	2.79 ± 0.13^{b}	2.50 ± 0.12^{ab}	2.02 ± 0.12^{a}
Cost of SoyaGold oil (ZK)	0	0.40 ± 0.21^{a}	3.70 ± 0.20^{b}	7.27 ± 0.19^{c}
Cost of Vitamin (ZK)	0	10.02 ± 0.45	9.94 ± 0.44	10.44 ± 0.43
Cost of DCP (ZK)	0	1.56 ± 0.07	1.55 ± 0.07	1.624 ± 0.07
TC (ZK)	0	31.439 ± 1.67	33.653 ± 1.66	38.54 ± 1.68
TR (ZK)	187.15 ± 17.56^{a}	333.27 ± 9.64^{b}	335.05 ± 9.64^{b}	338.15 ± 9.70^{b}
∏ (ZK)	187.15 ± 17.56^{a}	301.94 ± 9.65^{b}	301.44 ± 9.65^{b}	300.19 ± 9.71^{b}

Different superscripts in a row indicate significant difference ($P < 0.05$).

Table 4.17: Cost analysis of different combinations of lipid and protein diets fed to *O. andersonii* (mean ± SE)

Parameter	Unfed	10CL20CP	15CL20CP	20CL20CP	10CL30CP	15CL30CP	20CL30CP	10CL40CP	15CL40CP	20CL40CP
Cost of soya bean (ZK)	-	7.84±1.37^{a}	9.76±1.35^{a}	9.45±1.25^{a}	17.80±1.47^{b}	16.60±1.35^{b}	17.29±1.35^{b}	24.54±1.35^{bc}	22.47±1.37^{d}	27.26±1.35^{d}
Cost of maize bran (ZK)	-	4.10±0.22^{ef}	4.48±0.21^{f}	3.77±0.20^{e}	3.08±0.24^{d}	2.37±0.21^{c}	1.98±0.21^{c}	1.18±0.21^{b}	0.66±0.22^{ab}	0.31±0.21^{a}
Cost of SoyGold oil (ZK)	-	0.78±0.35^{a}	4.51±0.34^{c}	7.73±0.32^{d}	0.40±0.38^{a}	3.63±0.34^{bc}	7.01±0.34^{d}	0^{a}	2.96±0.35^{b}	7.06±0.34^{d}
Cost of Vitamin (ZK)	-	9.08±0.77	10.81±0.76	10.35±0.70	10.83±0.83	9.88±0.76	10.06±0.76	10.15±0.76	9.14±0.77	10.91±0.76
Cost of DCP (ZK)	-	1.41±0.12^{a}	1.68±0.12^{a}	1.61±0.11^{a}	1.68±0.13^{bc}	1.54±0.12^{b}	1.56±1.2^{b}	1.56±0.12^{cd}	1.42±0.12^{de}	1.70±0.12^{e}
TC (ZK)	0	23.23± 2.68^{a}	30.74± 2.68^{ab}	33.27± 2.55^{b}	33.79±2.89^{b}	33.90±2.78^{b}	36.18±2.83^{b}	37.30±2.68^{b}	36.32±2.78^{b}	46.17± 2.95^{c}
TR (ZK)	187.1±16.34^{a}	282.58±15.17^{b}	318.41±15.17^{bc}	280.05±14.44^{b}	360.82±16.34^{cd}	343.11±15.72^{c}	358.80±16.02^{cd}	360.21±15.17^{cd}	344.84± 15.72^{cd}	393.25±16.68^{d}
∏ (ZK)	187.15± 16.34^{a}	259.35±15.49^{b}	287.67±15.49^{bc}	246.79±14.75^{b}	327.03±16.69^{cd}	309.21±16.06^{cd}	322.62±16.36^{cd}	322.91±15.49^{cd}	308.53±16.06^{cd}	347.08±17.03^{d}

Different superscripts in a row indicate significant difference ($P < 0.05$).

Table 4.18: Multiple regression of the gross margin and, growth and feed parameters (only significant parametres are shown)

Independent variable	**Coefficient β**	**SE**	**Constant (Intercept)**	**r^2**	***P* – value**
BWG	13.063	0.093	107.315	0.993	0.001
SGR	-33.053	1.465			
AFCE	0.077	0.009			
Lipid level	-1.170	0.085			

4.5 Discussion

There were no significant differences ($P > 0.05$) in the interaction effect between protein and lipid in growth parameters (final weight (g), BWG (g) and SGR (% day^{-1})) and feed utilisation parameters (APERs and AFCE %) showing that both factors performed independently from each other. However, interaction effect was observed in the PLD (%) and PPD (%) showing that both lipid and protein depended on each other.

The growth of the unfed fish was significantly lower ($P < 0.05$) than the fish that was subjected to different combinations of protein and lipid. According to de Graaf *et al.* (1999), a reduction in somatic growth can lead to stunted growth of *O. niloticus*, resulting in fewer marketable sized fish that can be harvested. In the current study, none feeding in the unfed group might have resulted into stunting since the somatic growth was significantly lower ($P < 0.05$) than the fed *O. andersonii*.

Growth performance of *O. andersonii* was significantly ($P < 0.05$) affected by different combinations of dietary protein and lipid with the best final weight and body weight gain being achieved when the fish was fed with 20% lipid and 40% protein corresponding to 0.091g/Kcal (P:E) although this was not significant different ($P > 0.05$) from the combinations involving the 30% and 40% crude protein. A similar trend was observed in the SGR (%day^{-1}). This could be attributed to the high presence of crude protein in the feed providing much needed amino acids in building the body tissues (Hepher, 1988).

A polynomial regression equation ($Y = -0.0466x^2 + 1.4255x + 12.219$; $F = 5079$; $r = 0.988$) to describe the effect of dietary lipid on body weight gain showed 15.3% as optimal. Both APER and AFCE (%) were highest at 15% lipid. Studies by De Silva *et al*. (1991) on the hybrid *O. mossambicus* X *O. niloticus* found 18% lipid as optimal although they did not indicate the source of the lipid. The optimal dietary lipid for tilapia has been estimated to be less than 10% (Guillaume *et al*., 2001). Uys (1989) observed 10 – 12% as optimum lipid level for *C. gariepinus*. This is similar to what has been found in the current study on *O. andersonii* since the 10% lipid was not significantly different ($P > 0.05$) from the 15% and 20% lipid levels. In the current study no differences ($P > 0.05$) were observed in the growth parameters among the lipid levels in the fed groups. Similar results were observed by Hanley (1991) in supplementary feeds at levels of 5, 9 and 12% lipid. Therefore, soya bean based lipid requirements can be maintained as low as 10%. This confirms the low requirement of non-protein energy sources requiring the high dietary protein (Jauncey, 1998).

Guillaume *et al*. (2001) observed that the lipid optimal values are becoming obsolete since the values in salmonid feeds have continued to rise exceeding 30% probably due to high lipid digestibility coefficients that range between 93 – 98%. The hybrid tilapia *O. niloticus* x *Oreochromis aureus* showed satisfactory growth performance when dietary protein was decreased from 24 to 21% and dietary lipid level increased from 9 to 15% (Shiau and Huang, 1990; Ali, 2001). Similar results were found on the hybrid *Clarias* catfish *Clarias macrocephalus* x *C. gariepinus* by Jantrarotai *et al*. (1998). In their experiment growth performance was similar when dietary protein was decreased from 40

to 35% and lipid increased from 11 to 15%. Takeuchi *et al*. (1978) observed that the growth performance of trout is not affected even if the protein level is reduced to 35% provided that the lipid level is maintained at 18%. In this experiment 15.3% lipid was found to be optimal which is within the scope of the studies mentioned above.

Protein requirement is the first nutritional parameter to be determined for formulated feed production for newly established cultured fish species (Kim and Lee, 2009) due to cost implications and avoidance of water pollution through ammonia. Controlling the effect of lipid in the diets, 40% CP gave the highest final body weight and body weight gain although this was not significantly different ($P > 0.05$) from the 30% CP. In a study conducted by Gunasekera and Lam (1996) on *O. niloticus* brood stock found no significant difference ($P > 0.05$) in growth between the fish fed with the 20% CP and 30% CP. In the current study, the final body weights between the 20% CP and 30% CP were significant ($P < 0.05$) although the latter was not significant different ($P > 0.05$) from the 40% CP level. The variance would be attributed to the size and species used in the experiments. In the current study the experimental animal used was *O. andersonii* at juvenile stage while Gunasekera and Lam (1996) used brooding *O. niloticus*.

Polynomial regression equation ($Y = 0.318x + 12.797$, $F = 4773$, $r = 0.97$) showed that the relationship between the protein level and body weight gain was linear. This shows that the highest protein level (40%) used was not high enough to impose detrimental effects on the body weight gain of *O. andersonii*.

The total cost of producing fish at 40% CP was significantly higher ($P< 0.05$) than the 30% and 20% CP feed. Similarly the cost of producing the 20CL40CP feed was the highest. It is widely known that protein is the most expensive nutrient in the fish feeds due to high levels of inclusion in addition to the high cost of protein sources. The cost analysis clearly shows that GM is positively correlated to the BWG (g) and AFCE (%) but negatively related to SGR (%day^{-1}) and lipid level, and these were found to be contributing too strongly to the GM model (GM = 107.315 + 13.063BWG – 33.053SGR + 0.077AFCE – 1.17L). This means that the gross margin would decrease by ZK1.17 and ZK33.05 if lipid level and SGR (%day^{-1}) increased by 1% and 1%day^{-1}, respectively.

The 40% CP resulted into the highest final fish weight and economical returns. There were no significant differences ($P> 0.05$) in the fish growth rate at any lipid level used. The 20CL40CP treatment was not different ($P> 0.05$) from all other combinations with 30 and 40% CP. Gross Margin and TC were highest too at the highest lipid and protein combinations. This could be accredited to the fact that high quality feed cost more but produce high yield resulting into higher revenue. Oladejo (2010) reported similar results when the cost of *C. gariepinus* fingerlings was found to be proportionally related to revenue.

Data on the body composition of fish allows assessment of the efficiency of transfer of nutrients from feed to fish and also helps in predicting the overall nutritional status (Ali *et al.,* 2000). In most cases retention of energy and deposition of new tissue results in an increase in the weight of an animal, and the weight of young fish is usually a reliable

indicator of adequacy of the nutritional and management regimes. In the current studies, data on whole body composition indicated significant differences ($P< 0.05$) among treatments. The body protein of fish increased at the end of the experiment showing that fish growth was as a result of protein synthesis and tissue production and not only to fish weight only due to lipid deposition. Similar results were observed in *C. gariepinus* (Fafioye *et al.*, 2005). This is consistent with the feed utilisation data which showed high deposition of lipid at lower lipid inclusion level than at high levels.

In the current studies, there is no evidence to show that the levels up to 20% lipid sourced from soya bean to have an effect on the body lipid and protein. Similarly, crude protein of up to 40% did not affect the body crude protein and lipid. This is contrary to the study conducted on Tiger puffer (*Takifugu rubripes*) by Kim and Lee (2009). In their experiment there was a dose dependent response to dietary protein levels.

Biologically and economically, therefore, the combinations of 30% and 40% CP with any lipid level used in the experiment would be ideal in *O. andersonii* when using soya bean as the source of protein and lipid since BWG, final weight and GM were similar ($P> 0.05$) at these combinations. However, based on the cost of production of the feed, the feed 10CL30CP would be recommended since it was the least cost feed producing fish and income similar to all other 30% and 40% CP combinations. A follow up study is recommended with higher levels of protein above 40% in order to determine the maximum soya bean protein response of *O. andersonii*.

CHAPTER 5

EFFECT OF VARYING SOYABEAN PROTEIN AND LIPID ON REPRODUCTION OF *OREOCHROMIS ANDERSONII*

5.1 Introduction

The availability of quality fingerlings for stocking in aquaculture ponds has been reported as one of the key constraint to the development of aquaculture in Africa (Moehl and Halwart, 2005); since profitability of the aquaculture production depends on the availability of good quality fingerlings. Therefore, obtaining quality gametes and larvae becomes indispensable in modern aquaculture (Kucharezyk *et al*., 2008).

Gonadal developments are affected by certain essential dietary nutrients, especially in continuous spawners with short vitellogenic periods (Izquierdo *et al*., 2000) such as *O. andersonii*. Proteins and lipids, the main components of egg yolk, are considered to play a pivotal role in reproduction. Proteins act as a source of amino acids and reservoir of materials used during biosynthetic activities essential for early stages of embryogenesis (Metcoff, 1986; Khan, 2005). Lipids are the sources of essential fatty acids and play an important role in the development of the gonads. The fry depends on the nutritive constituents derived from the parents up to the time they exhaust the yolk since the development of fertilizable egg requires a substantial accumulation of nutritive resources for the development of the future embryo (Lokman *et al*., 2007). Therefore, it is important for brood stock fish to be fed with quality diets.

De Silva and Anderson (1995) pointed out that there is an optimum level of protein for reproduction although this is related to the growth of the fish species concerned. It has been recognized that diets for brood stock should be tailor made in order to ensure good quality as different fish species have different dietary requirement (Brooks *et al*., 1997). Brood stock nutrition of *O. andersonii* has not received much needed attention despite its importance in the aquaculture production. This is because the composition of the brood stock diets is known to influence on the reproduction and egg quality of several fish species (Brooks *et al*., 1997; Çoban*et al*., 2011).

5.2 Materials and method

The experiment was set up as described in section 4.2 and the treatments were as described in chapter 3. The fish stocked in the hapas is given in Table 5.1.

5.2.1 Egg size, fecundity, gonadosomatic, hepatosomatic and cardiosomatic indices

After 142 days thirty fish (15 females and 15 males) from each treatment were dissected and the gonads, heart and liver recovered before they were weighed to the nearest 0.01g. The stage of the gonads was identified and classified according to Balarin (1983) (Table 5.2).

Twenty gonads (10 males and 10 females) from each treatment were then put in individual vials with 10% formalin. Only ovaries in the ripe stage and that did not lose any eggs were preserved (Peña – Mendoza *et al.*, 2005).

Table 5.1: Stocking weight (g), SL and TL (mm) of *O. andersonii* used in the experiment (mean ± SE)

Treatment	**Unfed**	**10CL20CP**	**15CL20CP**	**20CL20CP**	**10CL30CP**	**15CL30CP**	**20CL30CP**	**10CL40CP**	**15CL40CP**	**20CL40CP**
Weight (g)	5.180 ± 0.214	4.747 ± 0.146	5.100 ± 0.188	4.943 ± 0.174	5.517 ± 0.201	4.907 ± 0.186	5.120 ± 0.163	5.080 ± 0.154	5.370 ± 0.219	5.067 ± 0.156
SL (mm)	50.233 ± 0.736	48.032 ± 0.643	50.067 ± 0.755	50.733 ± 0.811	51.767 ± 0.738	48.667 ± 0.908	49.767 ± 0.689	50.633 ± 0.939	50.170 ± 1.911	48.967 ± 0.712
TL (mm)	64.933 ± 0.897	63.000 ± 0.675	65.567 ± 0.900	65.500 ± 0.885	67.867 ± 0.900	63.667 ± 0.919	64.867 ± 0.822	65.567 ± 0.822	66.033 ± 1.039	64.167 ± 0.842

Table 5.2: Morphological description of gonadal maturation

Gonad maturity stage	Appearance of the male gonad	Appearance of the female gonad
Immature	Testes thin threadlike, flesh coloured, colourless to transparent.	Ovary threadlike, transparent and close to abdomen wall.
Inactive	Translucent testes, wider and generally longer than the ovaries in inactive female fish	Cream colour, translucent, elongated wider than testis. No oocytes visible opaque to translucent, occupy half of the visceral cavity. Few oocytes barely visible.
Inactive – Active		Opaque to translucent, occupy half of the visceral cavity. Few oocytes barely visible.
Active	Dull white/yellowish, thickened and elongated, about ¾ visceral cavity.	Ovary not yet swollen, but oocytes visible, yellowish with red hue.
Ripe	Cream white, distended fully over length of visceral cavity, milt evident if testes are cut	Yellow, green or orange eggs characteristic of species, large uniform size. Occupy all available space in the visceral cavity.
Ripe running	White/silvery, fully distended, milt runs freely under pressure	Ovary extremely swollen and eggs run under hand pressure or separate if ovary is cut.
Spent	Flesh/red colour shrunken with blood capillaries evident	Flaccid shrunken ovary, reddish with blood and small eggs discernible.

Source: Balarin (1983)

The preserved gonads were removed from the fixative formalin and put on the blotting tissue before they were put on the petri dish for egg counting. Gonadosomatic index, fecundity and cardio – somatic index, hepatosomatic index (HSI) (Shammi and Bhatnagar, 2002) were calculated as shown below:

$$\text{GSI (\%)} = \frac{\text{Weight of gonads (g)}}{\text{Weight of fish (g)}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 5.1$$

$$\text{Fecundity (\%)} = \frac{\text{No. of eggs}}{\text{Weight of fish (g)}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 5.2$$

$$\text{Cardio} - \text{somatic index} = \frac{\text{weight of heart (g)}}{\text{Weight of fish (g)}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 5.3$$

$$\text{Hepatosomatic index} = \frac{\text{weight of liver (g)}}{\text{Weight of fish (g)}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 5.4$$

The selected eggs were then taken to the calibrated eyepiece microscope for diameter measurement along two axes (long and short) since the eggs of *O. andersonii* were ellipsoid. The mean of the two axes represented the size of eggs. The number of eggs selected was based on the formula described in section 4.2.2. Each treatment was supposed to have 493 eggs measured for diameter on the microscope. However, since 10 females were sampled from each treatment 50 eggs were sampled from the each fish taking to 500 eggs per treatment.

Reproductive effort was measured by multiplying fecundity and egg size as given below (Duarte and Alcaraz, 1989).

Reproductive effort (RE) = fecundity x egg size…….5.5

5.2.2 Spawning performance

In order to study the spawning performance of *O. andersonii,* the females and males were separated for the period of 175 days in hapas as described in chapter 3. Each combination had six females and four males. Three females and one male were randomly selected and assigned to the different hapas and continued with the same feeding regime. Before the fish were mixed (males and females), they were checked for egg incubation in the mouth and interestingly treatments 10CL20CP, 20CL30CP, 15CL40CP and 20CL40CP had one female fish with eggs in the mouth. Ten eggs were incubated in the indoor hatchery with a recirculation system maintained at 28°C with the help of underwater plastic panel heaters (RL, 200N) immersed in the ground and overhead tank. The females that were incubating were excluded in the experiment.

5.3 Data analysis

Data collected and calculated on the reproductive performance of *O. andersonii* were analysed as described in section 3.6. In addition, multiple regression was performed in order to identify parameters that correlated and affected the reproductive characteristics of the fish. The model used is as follows:

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + \mathcal{E} \quad \text{.....5.6}$$

Where:

Y = Dependent variable (fecundity, GSI and egg size)

X_1 to X_4 = Independent variables

X_1 = Protein level (%)

X_2 = Lipid level (%)

X_3 = Weight of fish (g)

X_4 = interaction between protein and lipid levels if existed

b_1 to b_4 = regression coefficients

a = Constant

$\mathcal{E}$ = Residual

The stages of maturity of fish was analysed using the multinomial logistic regression models where size (SL), sex, protein, lipid and their combinations entered as factors and stages of gonads as dependant variables. In order to eliminate cells with zero frequencies, the gonadal stages were grouped into three categories. These were immature, inactive (inactive and inactive – active) and ripe (active, ripe and ripe running) only. The immature gonads were used as a reference group. The size (SL) was collapsed into 5mm intervals. Interactions between and among the factors were tested after the main effects were maintained in the model if significant ($P< 0.05$), and similarly factors not having significant ($P< 0.05$) effect were taken out from the model.

5.4 Results

Oreochromis andersonii (74.611%) were mature and this was significant ($P< 0.05$) at this size (24.477 ± 7.38g; mean ± SD). Although there were more mature males than females, Chi –square test showed no significant differences ($P> 0.05$) between sexes in terms of the reproductive status (Table 5.3).

Table 5.3: Number of immature and mature *O. andersonii* according to sex

			Maturity stage of the fish		
			Immature	**Mature**	**Total**
Fish sex	Female	Count	17	67	84
		Expected count	21.3	62.7	84.0
		% within fish sex	20.2	79.8	100.0
	Male	Count	32	77	109
		Expected count	27.7	81.3	109.0
		% within fish sex	29.4	70.6	100.0
Total		Count	49	144	193

χ^2 = 1.629, df = 1, *P*> 0.05

The protein and lipid combinations seemed to affect the reproductive status of *O. andersonii* as fisher's exact test (P = 0.039) showed significant differences (P< 0.05) among the treatments on the maturity of the fish under study (Figure 5.4).

Table 5.4: Number of mature and immature *O. andersonii* according to protein and lipid combinations

		Treatment										
		Unfed	**10CL20CP**	**15CL20CP**	**20CL20CP**	**10CL30CP**	**15CL30CP**	**20CL30CP**	**10CL40CP**	**15CL40CP**	**20CL40CP**	**Total**
Immature	Count	7	2	5	10	6	1	5	6	4	3	49
	Expected Count	3.3	4.8	5.1	6.3	4.3	5.1	5.1	5.1	4.8	5.1	49.0
	% within Maturity stage of the fish	14.3	4.1	10.2	20.4	12.2	2.0	10.2	12.2	8.2	6.1	100.0
Mature	Count	6	17	15	15	11	19	15	14	15	17	144
	Expected Count	9.7	14.2	14.9	18.7	12.7	14.9	14.9	14.9	14.2	14.9	144.0
	% within Maturity stage of the fish	4.2	11.8	10.4	10.4	7.6	13.2	10.4	9.7	10.4	11.8	100.0
	Count	13	19	20	25	17	20	20	20	19	20	193

Fisher's exact *P* = 0.039

A series of Mann Whitney U tests were used to separate the Mean Ranks. Significant differences ($P< 0.05$) were observed in the number of mature *O. andersonii* between the pairs of treatments (Unfed and 15CL30CP, Unfed and 15CL40CP, 10CL20CP and 20CL20CP, 20CL20CP and 15CL30CP, 10CL30CP and 15CL30CP, 15CL30CP and 10CL40CP) (Appendix 10.2). Although no significant differences ($P> 0.05$) were observed between the immature and mature fish in the unfed group, the mature fish were only 46.15%. In the fed fish, mature fish constituted over 50% with the treatment 15CL30CP recording the highest number of mature fish (95%) followed by 10CL20CP (89.47%), 20CL40CP (85%) and 15CL40CP (78.95%). Of the fed group fish subjected to the 20CL20CP diet recorded the least number of mature fish (60%) (Figure 5.1).

There were no significant differences ($\chi^2 = 6.520$, df = 3, $P = 0.085$) in the maturity of *O. andersonii* subjected to different levels of protein. However, mature fish represented 74.6% (Table 5.5). Although no significant differences ($\chi^2 = 7.185$, df = 3, $P = 0.066$) were observed in the reproductive status of the fish subjected to different levels of lipid, immature fish were only 25.3% (Table 5.6).

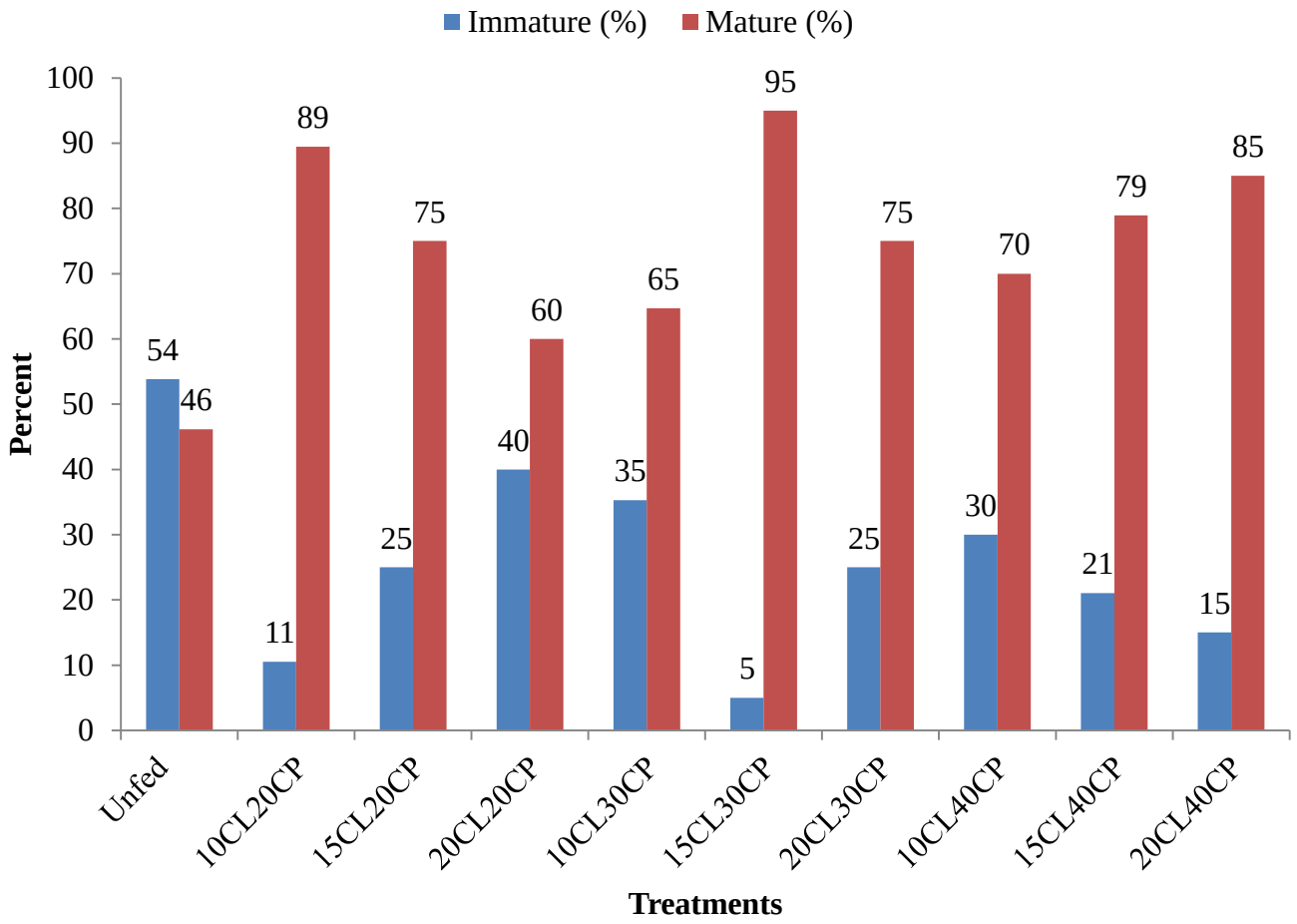


Figure 5.1: Percentage of sexual immature and mature *O. andersonii* (24.477 ± 7.380g; mean ± SD) according to treatment

Table 5.5: Cross tabulation of protein level and reproductive stage of fish

		Protein level (%)				Total
		0	**20**	**30**	**40**	
Immature	Count	7	17	12	13	49
	Expected count	3.3	16.2	14.5	15.0	49.0
	% within maturity stage of the fish	14.3	34.7	24.5	26.5	100.0
Mature	Count	6	47	45	46	144
	Expected count	9.7	47.8	42.5	44.0	144.0
	% within maturity stage of the fish	4.2	32.6	31.3	31.9	100.0
Total	Count	13	64	57	59	193

χ^2 = 6.520, df = 3, *P* = 0.085

Table 5.6: Cross tabulation of lipid level and reproductive stage of *O. andersonii*

			Lipid level (%)				Total
			0	**10**	**15**	**20**	
Maturity stage of the fish	Immature	Count	7	14	11	17	49
		Expected count	3.3	14.2	15.2	16.2	49.0
		% within maturity stage of the fish	14.3	28.6	22.4	34.7	100.0
	Mature	Count	6	42	49	47	144
		Expected count	9.7	41.8	44.8	47.8	144.0
		% within maturity stage of the fish	4.2	29.2	34.0	32.6	100.0
Total		Count	13	56	60	64	193

χ^2 = 7.185, df = 3, *P* = 0.066

To explore the influence of lipid and protein on the reproductive characteristics of *O. andersonii* a Two – Way analysis of Variance (ANOVA) (General Linear Model) was conducted. This was done by analysing the effects of lipid, protein and the interaction on the GSI, egg size and fecundity in *O. andersonii*. Significant ($P< 0.05$) dietary protein by lipid level interaction was found among diets when protein level and lipid were used to examine the fecundity, GSI, egg weight and egg diameter. This suggests that the effect of lipid depended on protein on the reproductive characteristics. Splitting data according to lipid levels, significant differences ($P< 0.05$) were observed in all the four reproductive characteristics. Since there were fewer than two groups for the unfed group, it was excluded in the analysis.

Duncan's Multiple Range Test showed that the means for fecundity were only significant ($P< 0.05$) at 15% and 20% with the highest fecundity recorded at 15CL20CP diet though not significant different ($P> 0.05$) from the 15CL40CP diet. The lowest fecundity was observed at 20CL20CP (Table 5.7).

Table 5.7: Fecundity of *O. andersonii* fed different protein levels (mean ± SE)

	Protein level (%)		
Lipid level (%)	**20**	**30**	**40**
10	522.062±83.627	546.777±68.120	647.168±170.883
15	653.323±27.944^{b}	430.706±32.396^{a}	556.433±20.370^{b}
20	377.963±64.367^{a}	464.761±45.656^{a}	612.722±32.267^{b}

Different superscripts in a row indicate significant difference ($P < 0.05$).

Similarly the means for GSI were only significant at 15% and 20% with the highest recorded at 15CL20CP diet though not significant different (P> 0.05) from the 15CL40CP diet. The lowest GSI was observed at 20CL20CP (Table 5.8).

Table 5.8: Gonadosomatic index of *O. andersonii* fed levels of protein (mean ± SE)

	Protein level (%)		
Lipid level (%)	**20**	**30**	**40**
10	1.927±0.283	2.496±0.186	1.547±0.716
15	2.637±0.177^{b}	1.778±0.160^{a}	2.531±0.294^{b}
20	1.011±0.139^{a}	1.823±0.248^{b}	2.378±0.181^{b}

Different superscripts in a row indicate significant difference (P < 0.05).

Significant differences (P< 0.05) were observed at all levels of lipid for egg weight. At 15% lipid level, the highest egg weight was observed at 40% protein. The diets with 20% and 30% protein levels were not significant different (P> 0.05) from each other (Table 5.9).

Table 5.9: Egg weight (mg) of *O. andersonii* fed levels of protein (mean ± SE)

	Protein level (%)		
Lipid level (%)	**20**	**30**	**40**
10	3.490±0.295^{a}	5.430±0.390^{b}	3.000±0.924^{a}
15	3.790±0.154^{a}	4.480±0.400^{ab}	5.540±0.457^{b}
20	3.440±0.280^{a}	4.020±0.469^{a}	3.730±0.176^{b}

Different superscripts in a row indicate significant difference (P< 0.05).

Significant differences ($P< 0.05$) were observed at all levels of lipid for egg diameter. The highest egg diameter was observed in fish eggs fed with 15CL40CP diet. At 10% lipid level, the highest egg diameter was observed at 30% protein level while 40% protein at 15% level (Table 5.10).

Table 5.10: Egg diameter (mm) of *O. andersonii* fed levels of protein (mean ± SE)

	Protein level (%)		
Lipid level (%)	**20**	**30**	**40**
10	0.116 ± 0.004^{ab}	0.131 ± 0.005^{b}	0.106 ± 0.001^{a}
15	0.147 ± 0.007^{ab}	0.081 ± 0.009^{a}	0.167 ± 0.014^{b}
20	0.047 ± 0.007^{a}	0.103 ± 0.012^{b}	0.106 ± 0.007^{b}

Different superscripts in a row indicate significant difference ($P< 0.05$).

Multiple regression analysis showed that protein level only affected strongly GSI, while fecundity was strongly affected by lipid and fish weight. Egg weight was affected strongly by fish weight, protein and the interaction between lipid and protein. Similarly, fish weight, protein and interaction between protein and lipid affected strongly the egg diameter (Table 5.11).

Table 5.11: Multiple regression of reproductive characteristics of *O. andersonii* subjected to protein and lipid combinations

Dependant variables	**Independent variables**	**Coefficient β**	**SE**	**Constant (Intercept)**	**r^2**	***P* – value**
GSI	Protein (%)	0.24	0.007	1.411	0.03	0.001
Fecundity	Lipid (%)	0.012	0.003	2.744	0.11	0.0001
	Fish weight (g)	-0.004	0.001			
Egg weight (mg)	Fish weight (g)	1.96×10^{-5}	0.001	0.001	0.13	0.001
	Protein (%)	0.001	0.001			
	Protein*lipid	5.00×10^{-6}	0.001			
Egg diameter (mm)	Fish weight (g)	0.001	0.0001	0.026	0.11	0.0001
	Protein (%)	0.004	0.001			
	Protein*lipid	0.0001	0.001			

The likelihood ratio shows the contribution of each variable to the model and it revealed that sex and fish size (SL) were the only factors that significantly ($P< 0.05$) affected the advancement of maturity in *O. andersonii*. Protein, lipid and their interactions and the combinations were not significant ($P> 0.05$) and therefore were removed from the model.

Female fish were more likely (odds ratio = 3.9, $P< 0.05$ for inactive and 4.1, $P< 0.05$ for ripe) to be in the advanced maturity stage than males. This shows that females

mature earlier than females. Large sized fish were more likely to be in the advanced maturity stage than small fish. This shows that fast growing fish are more prone to mature early than slow growing *O. andersonii* (Table 5.12).

Table 5.12: Logistic regression on likelihood of sex and fish size on maturity stage

Sex	**Maturity stage**	**B (coefficient)**	**S.E**	**Wald**	**Exp (B)**
Female					
	Inactive	1.37	0.476	8.272*	3.935
	Ripe	1.41	0.543	6.739*	4.096
Fish size (SL) (mm)					
60.0 – 65	Inactive	-39.006	0.000	-	1.15×10^{-17}
65.1 – 70		-19.100	1.614	140.067*	5.07×10^{-9}
70.1 – 75		-18.601	1.575	139.424*	8.35×10^{-9}
75.1 – 80		-17.890	1.573	129.349*	1.70×10^{-8}
80.1 – 85		-17.490	1.521	132.224*	2.54×10^{-8}
85.1 – 90		-17.939	1.532	137.154*	1.62×10^{-10}
90.1 – 95		-16.085	1.155	193.799*	1.03×10^{-7}
60.0 – 65	Ripe	-41.568	0.000	-	8.85×10^{-19}
65.1 – 70		-22.120	1.327	277.711*	2.47×10^{-10}
70.1 – 75		-23.021	1.361	286.106*	1.01×10^{-10}
75.1 – 80		-20.466	1.185	298.417*	1.29×10^{-9}
80.1 – 85		-21.193	1.168	329.163*	6.25×10^{-10}
85.1 – 90		-20.256	1.113	331.201*	1.59×10^{-9}
90.1 – 95		-17.354	0.000	-	2.91×10^{-8}

***significant**

Spawning performance

The eggs retrieved from the females without the males did not hatch and all died after 24 hours. For purposes of expressing data, the number of fish spawned was expressed as a percentage of the fish stocked for the particular treatment. A one way repeated ANOVA was conducted to compare scores on the percentage of fish that did not

spawn and the number of eggs retrieved in the mouth of fish with statistics test at 14, 28, 42 and 56 days after aggregating the number of fish. There was no significant interaction ($P > 0.05$) observed between protein and lipid in the number of eggs incubated in the mouth of the fish. Therefore, follow up analysis concentrated in the main effects.

There were no significant ($P > 0.05$) effect for time, Wilks Lambda = 0.212, F (3, 3) = 3.71, $P > 0.05$) for protein (Table 5.13).

Table 5.13: Descriptive statistics for fish (%) that did not spawn based on protein.

Period (days) after stocking	Protein (%)	Mean (%)	Std. Error	N
14	0*	37.700	-	1
	20	31.767	7.342	3
	30	23.633	3.201	3
	40	39.633	16.000	3
28	0*	25.00	-	1
	20	21.900	1.769	3
	30	25.133	5.787	3
	40	23.500	4.731	3
42	0*	18.800	-	1
	20	24.133	2.316	3
	30	24.867	5.787	3
	40	14.667	4.879	3
56	0*	18.800	-	1
	20	22.133	8.235	3
	30	26.367	3.200	3
	40	22.233	8.319	3

*Standard error not possible since it was not replicated

Similarly, no significant ($P > 0.05$) effect were observed for time, Wilks Lambda, Wilks Lambda = 0.616 F (3, 3) = 0.623, $P > 0.05$) for lipid (Table 5.14).

Table 5.14: Descriptive statistics for fish (%) that did not spawn based on lipid level

Period (days) after stoking	Lipid level	Mean	Std. Error	N
14	0*	37.700	.	1
	10	37.067	15.134	3
	15	29.667	10.661	3
	20	28.300	10.136	3
28	0*	25.000	.	1
	10	24.500	6.855	3
	15	22.867	1.890	3
	20	23.167	3.744	3
42	0*	18.800	.	1
	10	16.933	7.283	3
	15	22.500	5.956	3
	20	24.233	5.254	3
56	0*	18.800	.	1
	10	21.467	2.857	3
	15	24.967	7.177	3
	20	24.300	9.734	3

*Standard error not possible since it was not replicated

There was a general decline in the number of eggs collected over time (Figure 5.2a and 5.2b). When using an ANOVA with repeated measures with sphericity assumed, the mean scores for egg number differed significantly (F (3, 8.993) = 10.381), $P < 0.05$) between different time points. Post hoc tests using Bonferroni correction revealed that the number of eggs collected after 14 days (457 ± 8) were significantly

higher than the collections done at 28 days (404 ± 9), 42 days (409 ± 6) and 56 days (391 ± 7). No significant differences ($P > 0.05$) were observed in the fed group in both nutrients.

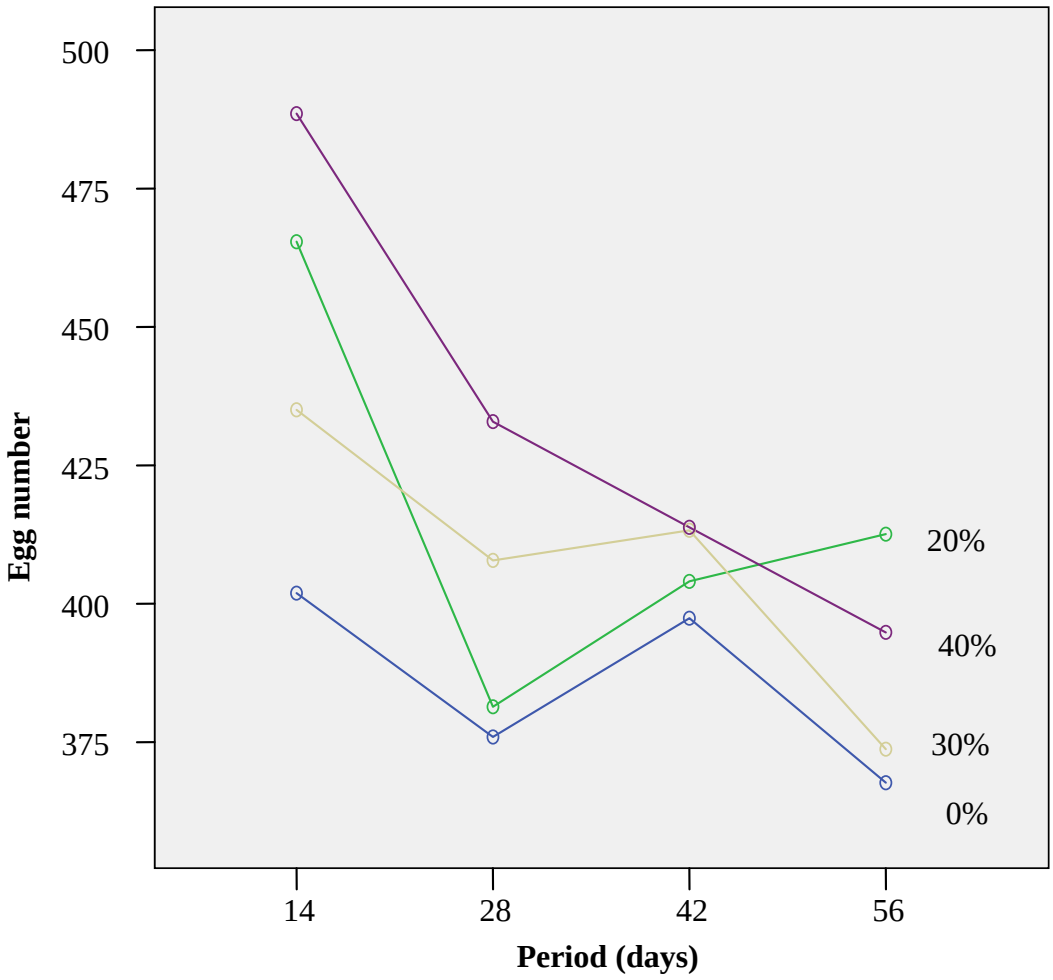


Figure 5.2a: Egg production trend over a 56 days period based on protein level

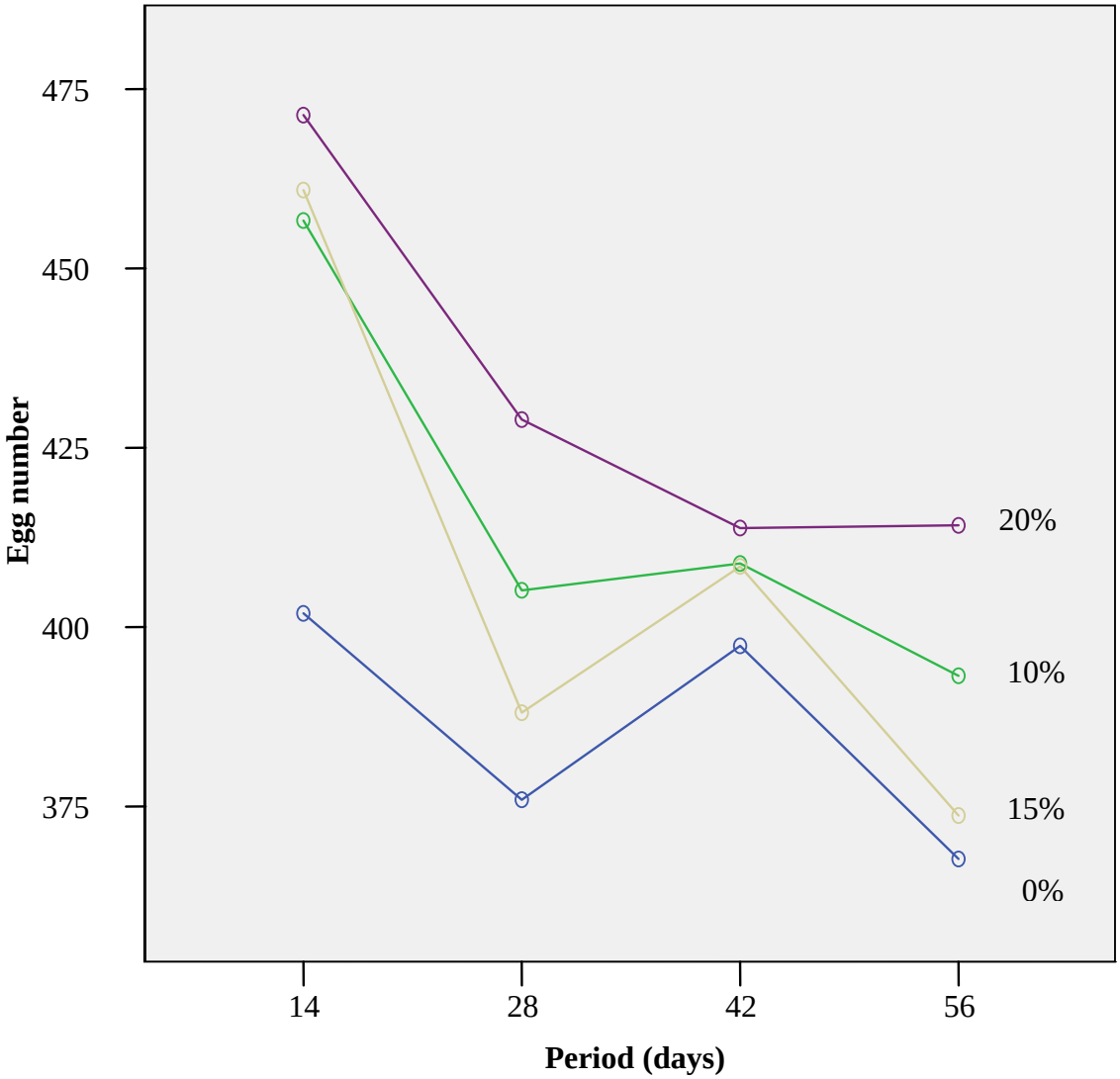


Figure 5.2b: Egg production trend over a 56 days period based on lipid level

5.5 Discussion

Gonadosomatic Index is an important reproductive parameter in fisheries and aquaculture sciences. However, it has been argued that it is suitable only to fish of similar sizes (Cayré and Laloë, 1986). In this experiment, the fish used had similar (P> 0.05) weights justifying the use of this maturity index. There have been no reports of GSI of *O. andersonii* subjected to two nutrients (protein and lipid) simultaneously. A significant interaction was found between lipid and protein. In an experiment conducted by Sink *et al*. (2010), a significant dietary protein by lipid was

observed among the diets when the protein and lipid sources were used to examine the free amino acids (FAA). In the current study, GSI and fecundity were highest when the lipid and protein from soya bean were fixed at 15% and 20%, respectively.

However, in terms of egg size (diameter and weight) the combination 15CL40CP seemed to maximize the egg diameter and weight. The results show that GSI and fecundity can be maximized at 20% protein and 15% lipid levels but that does not maximize the egg size. Sink *et al*. (2010) concluded that the brood stock feed for channel catfish should contain at least 35% crude protein and 10% supplemental lipid. Kithsiri *et al*. (2010) observed that in guppy females fed on higher levels of protein developed heavier gonads. In the current experiment, egg size was maximized at the highest protein level used in the study. Egg size is an important reproductive parameter since larger egg size results into larger fry ensuring greater survival (Seghal and Toor, 1991; Kithsiri *et al*., 2010).

Multiple regression analysis revealed the GSI, fecundity and egg size to have been affected by lipid, protein and fish weight. However, there was a greater variability in all the reproductive characteristics studied. The variability indicates the existence of wide range of reproductive tactics involving the allocation of resources to the reproductive effort (Duarte and Alcaraz, 1989). Alonso – Fernández *et al* (2014) found similar results in *Coris juris* an indeterminant spawner similar to the fish species under study. They speculated that the lower predictive power is as a result of of using batch fecundity which introduces another level of variation (with batch variation). In addition, it also shows the unsynchronization of egg development (plurimodal spawner) in *O. andersonii* imposing difficulties in spawn collection

synonymous to tilapia fish. This also is confirms the irregularity in the spawning intervals of the female fish as each time of the collection resulted in spawn of different stages and sizes.

Logistic regression shows that females of *O. andersonii* mature early than males. This is consistent to what Salerno *et al*. (2001) found on Bluefish, *Pomatomus saltatrix*. In their experiment females matured at 1.1 years against the 1.2 years for males. Similar results were reported by Shalloof and Salama (2008) in *O. niloticus* who found that females matured earlier than males. This could be attributed to the utilization of the energy in males for somatic growth while for reproduction in females (Boliver *et al*., 1993; Shubha and Reddy, 2011). The results, however, are contrary to what Al Hafedh *et al.* (1999) found on *O. niloticus* and Hatikakoty and Biswas (2002) on *O. mossambicus*. In their experiment males matured earlier than females. The results of the experiment show that bigger fish were more prone to mature early than smaller sizes. This is consistent with Al Hafedh *et al.* (1999) who found that fast growing fish mature earlier than slow-growing fish. This possess a paradox phenomenon in tilapia growth since improved management entails fast growth rate but accelerating maturity a stage at which growth is depressed.

There was a general decline in the number of eggs incubated over a period of 56 days. This could be explained by the diminishing resources of the female fish a phenomenon described by Hsiao *et al*. (1994). Similar results were observed by De Silva and Radampola (1990) who observed a reduction in egg production when *O. niloticus* was subjected to practical feed diets with varying levels of protein between 20 and 35%. However, Gunasekera *et al*. (1996) found fecundity of *O. niloticus* to be

increasing when fed with 20% and 30% protein diets. However, the opposite was observed at 10% protein level. The variation with the current study would be due to differences in the experimental design, fish species and size.

Sequential hermaphrodites (fish first functions as a female and then as male) depend on social factors rather than abiotic. This may be due to disappearance of the dominant sex or by change of sex ratio (Wotton, 2000). In the current experiment, the fish were kept separately according to sex but one of the female in the group of three spawned. Therefore, the spawning of the eggs in the absence of males would be attributed to the possibility of one female functioning as a male due to the absence of the normal male. Similar behaviour was observed by Gunasekera *et al.* (1996b) on *O. niloticus*.

The study shows that fecundity and GSI could be maximized at 15CL20CP while the egg size at the 15CL40CP combinations. However, incubation was maximized at the highest protein (40%) and lipid (20%) levels used in the experiment. The experiment also shows that female's *O. andersonii* can spawn without the presence of males. Furthermore, the study shows that females mature early than males. In terms of size fast growing fish are more prone to mature earlier than slow growing *O. andersonii*.

CHAPTER 6

EFFECT OF 17α – METHYL TESTOSTERONE ON THE GROWTH, REPRODUCTION, LIVER, HEART AND HAEMATOLOGY OF *OREOCHROMIS ANDERSONII*

6.1 Introduction

Hormonal use in aquaculture has been successful on reproduction and in single sex production. This is because production traits are not similar in both sexes in fish species; therefore, production can be improved by adopting mono – sex culture technique by either involving the male or female fish depending on the superiority of the growth performance (Hossain *et al*., 2002). Furthermore, reproduction in some fish species such as *Clarias gariepinus* is impaired in captivity; therefore hormones are used to induce the fish in order for them to reproduce in captivity or outside their normal breeding period.

While hormones have been used as growth promoters in the livestock industry, results in fish seem to be contradictory. For instance the experiments conducted by Soto (1992) on *O. niloticus* and Hossain *et al*. (2002) on *C. gariepinus* did not show any evidence that growth is enhanced when androgens are administered. However, according to Robles – Basto *et al.* (2011) the use of the androgenic hormone in sex reversal has anabolic effect that enhances growth and protein synthesis, resulting into a greater muscle mass gains.

The nutritive requirements of the developing eggs are mainly the yolk proteins which are laid down during the vitellogenesis, the major growth phase known to be under

the gonadotropin and estrogen control (Nagahama *et al*., 1995). The increase in previtellogenic oocytes in *A. australis* when administered with 11 – KT *in vivo* (Rohr *et al*., 2001) implicates the functional role of androgens in reproduction. However, the use of such hormones has not been tried on *O. andersonii* from the practical point of view.

Haematological characteristics have been widely used in clinical diagnosis of human and domestic animals (Soivio and Okari, 1976). They have been described to be indispensable parameters in evaluating physiological status of the fish (Vázquez and Guerrero, 2007). These indices can further be used to detect stress condition such as exposure to pollutants, hypoxia, transportation, anaesthetic and acclimation (Gabriel *et al*., 2012).

Blood is sensitive to environmental pollutants (Savari *et al*., 2011) and will reveal conditions within the body of the fish long before there is any visible sign of disease (Fernades and Mazon, 2003; Gabriel *et al*., 2012). In fact they have been frequently been used as indicators of stress in sturgeons (Falahatkar *et al*., 2009; Zarejabad *et al*., 2009; Hasanalipour *et al*., 2013). This is because haematological parameters are closely related to the response of the animal to the environment, an indication that the environment in which the fish lives can exert some influence on the blood parameters (Gabriel *et al*., 2004). However, haematological parameters vary with age, sex, activity of the fish and environment (Kapila *et al*., 2000). Although haematological parameters are a useful tool in fish physiology, literature in this area has been isolated and where available is usually incomplete (Summarwar, 2012).

According to Hrubec *et al.* (2000) and El – Hawarry (2011) although Tilapia are the second most frequently cultured fish species in the world, there are few reports of normal blood values. Blood parameters have proved to be valuable tools in providing information on metabolic disorders, deficiencies and chronic stress before any clinical symptoms can appear (El – Hawarry, 2011). Measurement of albumin, globulin, and total protein in serum or plasma is of considerable diagnostic value in fish, as it relates to general nutritional status of the fish (Schaperclaus *et al*., 1992). Lactate dehydrogenase (LDH) is an important enzyme in evaluating liver intoxication. It is known to occur in glycolysing cells and plays an important role in ammonia detoxification (Adham *et al*., 2002). It is an enzyme that helps the fish that produces energy (Hasheesh *et al*., 2011). Creatinine and uric acid levels indicate the kidney function (Abdel – Tawwab and Wafeek, 2008). Cholesterol is a chemical compound that is naturally produced by the body and is a combination of lipid (fat) and steroid. Cholesterol is a building block for cell membranes and for sex hormones like estrogen and testosterone. About 80% of the cholesterol is produced by the liver (Hasheesh *et al*., 2011).

The study was conducted to determine the effect of MT on the growth, reproduction, liver, heart and haematology of *O. andersonii*. The cost implication of using MT as a growth promoter in *O. andersonii* was also evaluated.

6.2 Materials and methods

6.2.1 Growth and reproduction

6.2.1.1 Experimental fish

The fish were seined from the 2, 500m^2 and conditioned in pond cage as described in Chapter 3. After three weeks the fish were scooped and put in the buckets and transported to the concrete tanks for commencement of the experiment.

6.2.1.2 Preparation of the concrete tanks and experimental design

Eight outdoor concrete tanks (10 x 5 x 0.7m) were drained, cleaned and allowed to dry for three days (Figure 6.1). Water was then pumped into them up to the depth of 40cm leaving 30cm freeboard to avoid the spilling of water since the water was receiving the feed with the hormone. In addition, the outlet pipes were sealed with plastics. To reduce the growth of any phytoplankton, fertilizers and lime were not applied.

After seven days the tanks were stocked with *O. andersonii* at 1 fish/m^2 randomly in a Complete Random Design (CRD). Each treatment was replicated twice. No significant differences (P> 0.05) were observed in the fish stocked among the all treatments (Table 6.1). The tanks were then covered with netting material to prevent predatory birds.

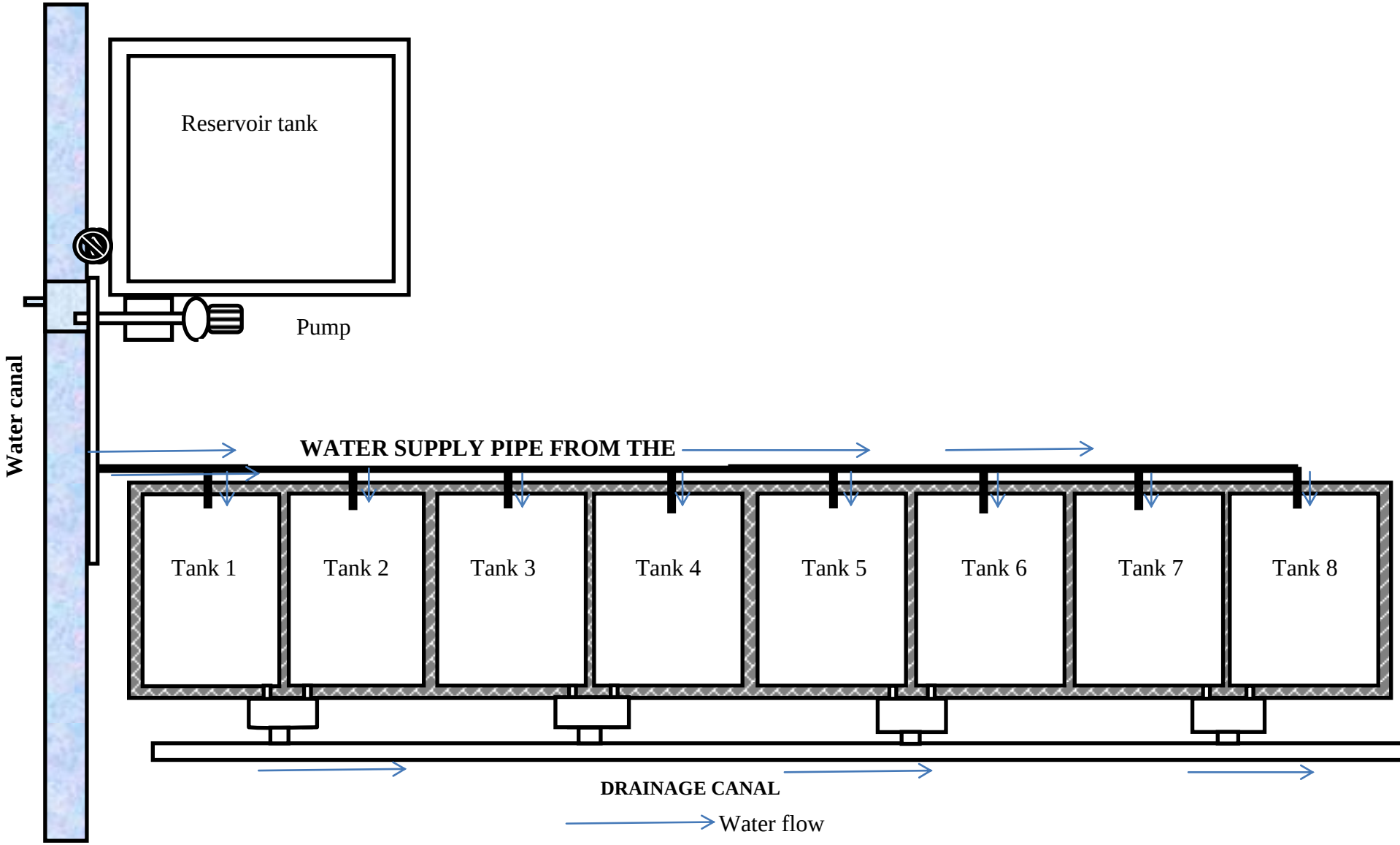


Figure 6.1: Schematic diagram of the experimental tanks

Table 6.1: Weight and length of the *O. andersonii* stocked in concrete tanks (mean ± SE)

Treatment	**0 mgMT/kg**	**40 mgMT/kg**	**60 mgMT/kg**	**90 mgMT/kg**
Weight (g)	8.705±0.580	7.995±0.461	8.175±0.473	8.983±0.582
SL (mm)	60.650±1.603	58.900±1.284	60.200±1.198	61.475±1.342
TL (mm)	77.050±2.130	74.150±1.694	75.675±1.616	78.950±1.800

6.2.1.3 Preparation of experimental feed

Isonitrogenous (30%) and isocaloric (4.02 kcal/g) diet was prepared using WinFeed 2.8 package after proximate analyses of ingredients (soya bean cake and maize bran) as described in Chapter 3. Three levels of MT (Aquaculture Solutions Limited, South Africa) of 40 mg/kg, 60 mg/kg and 90 mg/kg were included in the feed as treatments and hormone free feed as a control (Table 6.2). The MT was dissolved in 95% ethanol at the concentration of 15ml per kg of feed (Teichert – Coddington *et al*., 2000). The feed and hormone were then mixed in the dish until a homogeneous sample was achieved. The feed was then dried under direct sunlight for three hours to allow for the evaporation of the ethanol before they were taken on the pelleting machine attached with 3.2mm die. The pellets were then taken outside on racks for sun drying. The feed was stored in a chiller (Sanyo medicool) at 8°C till used.

Table 6.2: Composition of experimental diets (%) used to feed *O. andersonii*

Ingredients	**Dietary 17α – Methyl testosterone levels**			
17α – MT mg/kg feed	Control (0)	40	60	90
Soya bean cake (%)	56.0	56.0	56.0	56.0
Maize bran (%)	36.0	36.0	36.0	36.0
SoyaGold oil (%)	5.9	5.9	5.9	5.9
[1]Vitamin premix (%)	1	1	1	1
[2]DCP (%)	1	1	1	1
Proximate analysis				
Moisture (%)	13.9	13.9	13.9	13.9
Crude protein (%)	30	30	30	30
Crude fat (%)	15	15	15	15
Crude ash (%)	3.9	3.9	3.9	3.9
Carbohydrates (%)	37	37	37	37
Crude fibre (%)	6.4	6.4	6.4	6.4
Gross energy Kcal/g	4.02	4.02	4.02	4.02

[1]Vitamin stress pack (100g): Retinol 2, 000, 000 *I.u*; Cholecalciferol 300, 000 *I.u;* Tocopherols 3000 *I. u;* Menadione 300mg; Ascorbic acid 3, 000mg; Riboflavin 500mg; Niacin 2, 500mg; Pantothenic acid 1, 000mg; Cobalamines 3mg; Pyridoxine 200mg; Folic acid 50mg and Thiamine 200mg.

[2]D-calcium phosphate

6.2.1.4 Feeding and rearing

The fish were provided with the prepared feed twice (10:00 and 15:00 hours) daily except on Sundays calculated at 5% live body weight. Survival was monitored and all those that died in the first week of the experiment were replaced. Fortnightly, a total of 160 fish (20 from each tank or 40 from each treatment) were weighed and lengths (SL and TL) measured according to the procedures described by Skelton (2001) after the fish were anaesthetized in 5g/l crude clove (Unilever South Africa Foods (Pty) Ltd) to reduce stress. The experiment lasted for 119 days starting from 3rd January 2011. At the end of the experiment all the fish were caught and their weights and lengths (SL and TL) determined. In addition, 20 fish (10 of each sex) were taken to the laboratory for gonad and liver extraction. Upon extraction of the gonads, the maturity stage was determined according to Balarin (1983) before being weighed on a digital scale (CS – 58 – II). The gonads were then fixed in 10% formalin in individual vials for later egg counting and diameter determination on an ocular scale on the microscope after blotting them on paper.

Further 20 fish were killed and their gonads (10 of each sex) from every treatment were prepared for the histological examination by fixing them in 10% formalin solution. They were then embedded in paraffin wax after dehydration in series of methanol (70, 80, 90, and 100%) and sectioned in 3 µm before they were stained with Haematoxylin and Eosin. The sections were then mounted on slides with DPX mountant. The slides were then examined on the Olympus microscope (150 x power) and a picture taken with Kodak camera.

6.2.2 Liver, heart and haematological studies

Sixty four (64) *Oreochromis andersonii* (females = 115 ± 14.228g and males = 194.100 ± 25.909g; mean ± SD) were evenly allocated to four different hapas (size) installed in outdoor concrete tanks (10 x 5 x 0.7m) as described in section 6.2.1.2 and shown in Figure 6.1 in a sex ratio of 1:1. The fish were fed the feed described in sections 6.2.1.3 and 6.2.1.4.

On the 30th day, twelve fish from each treatment were carefully caught and anaesthetised in crude clove (5g/l of deionised water) before they were weighed and measured according to Skelton (2001). The fish were then wiped off water using a hand towel before their left gills punctured across with the blade. The fish were then tilted with the gill portion facing the vacutainer (purple top) collecting the oozing blood. Six fish samples (Figure 6.2) from each treatment were bled and 2ml blood collected from each fish and put in a separate vacutainer with an anticoagulant dipotassium ethylenediamine tetraacetic acid (EDTA) for haematological analysis. The parameters determined included White Blood Cells (WBC), Lymphocytes (LYM), Monocytes (MON), Granucytes (GRA), Red Blood Cells (RBC), Haemoglobin (Hb), Haematocrit (Ht), Mean Corpuscular Volume (MCV), Mean Corpuscular Volume Haemoglobin (MCH), Mean Corpuscular Volume Haemoglobin Concentration (MCHC), Red Blood Cell Distribution Width (RBCDW), Platelets (PLT), Mean Platelets Volume (MPV), Platecrit (PCT) and Platelets Distribution Width (PDW). The vacutainers were immediately put on ice cubes before taken to the laboratory. In the laboratory, the blood was mixed on a roller mixer and analysed on a Horiba ABX Micros 60 Cs according to the procedures outlined by the

manufacturer. A similar procedure was conducted for plasma chemistry although the blood was put in BD vacutainers (without EDTA) and was not put on the ice. In the laboratory the samples were analysed on an automated chemistry analyser (Olympus AU 400) using the standard procedures. Blood chemistry parameters determined included urea, creatinine, uric acid, total biririubin, lactate dehydrogenase (LDH), total protein, cholesterol, triglycerides and globulins. However, globulins were calculated from the difference between total protein and albumin values (Hrubec *et al.,* 2000).

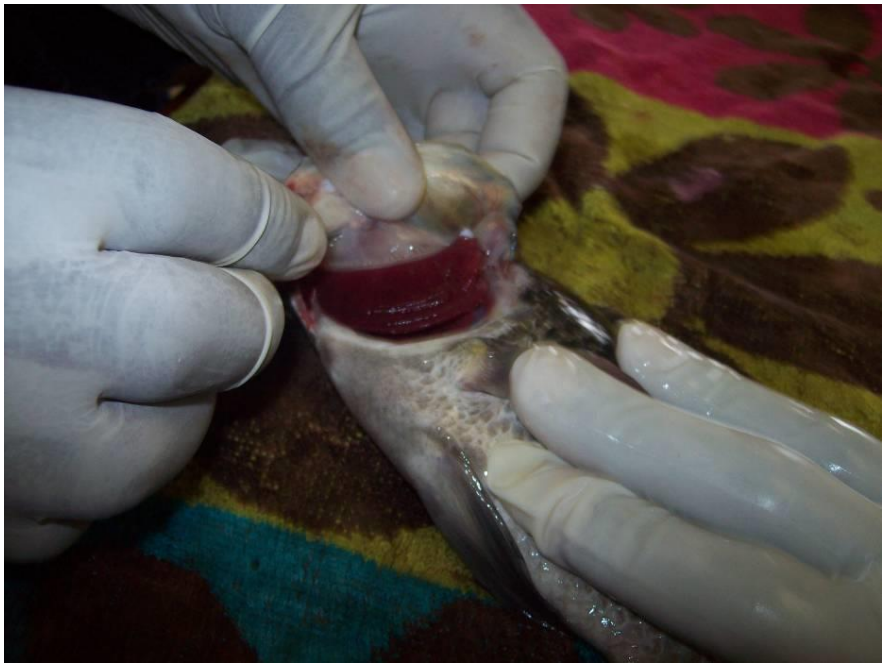


Figure 6.2*:* Gill of *O. andersonii* being cut to collect blood for haematology analysis

The liver and heart were then recovered from the fish and weighed on Citizen CY 204 model to the nearest 3 decimal places before they were preserved in 10% formalin for histological analysis as described for gonads in section 6.2.1.4.

6.2.3 Water quality

Water quality parameters were determined as in section 3.5.

6.3 Data analysis

Data were analysed as described in section 3.6 and growth parameters calculated as indicated in section 4.3 while reproductive, haematology, hepatosomatic and cardiosomatic indices as described in section 5.2.1.

Simple gross margin analysis was performed to determine the cost effectiveness of the prepared diet incorporated with MT. It was assumed that all other operating costs remained constant and only the variable cost of MT was used in calculations using the price (ZK58, 964.18/g) at which the MT was imported from Aquaculture Solutions of South Africa. The final weights were assumed to be the harvest weight of fish at the prevailing market price of ZK12, 000.00. The following key economic indicators were computed according to Faturoti and Lawal (1986) and Jolly and Clonts (1993).

$Y = P_{MT}Q_{MT}$ ----------i

Where Y = Total Cost (TC) of prepared diet incorporated with MT level (0, 40, 60 and 90 mg/kg); PMT = Unit cost of MT; Q_{MT} = Quantity of MT used at given level (0, 40, 60 and 90 mg/kg).

TR = P*FW----------6.1

Where; TR = Total revenue; P = price of fish; FW = final fish weights

$\prod = TR - Y$ ----------6.2

Where; $\prod$ is gross margin, Y and TR as described in equations i and ii above

$\prod R = \frac{\prod}{TR}$----------6.3

Where; $\prod R$ is gross margin ratio, Y and TR as described in equations ii and iii above

6.4 Results

6.4.1 Growth performance

Generally there was an increase in live body weight over time for the fish cultured (*O. andersonii*) in all treatments (Figure 6.3).

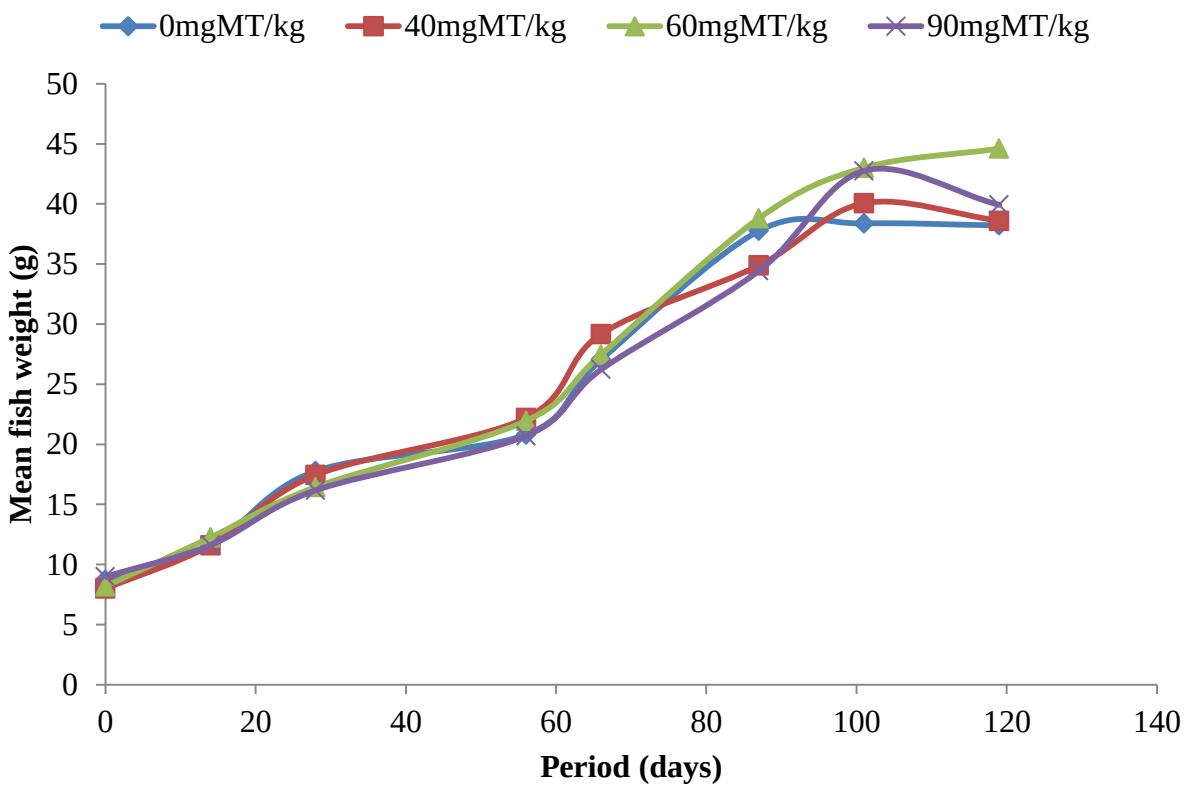


Figure 6.3: Growth trend of *O. andersonii* fed on MT for 119 days

However, the incremental growth in all the treatments was depressed after 87th day with the exception of the 90 mgMT/kg which showed the loss in weight after 101 days (Figure 6.4).

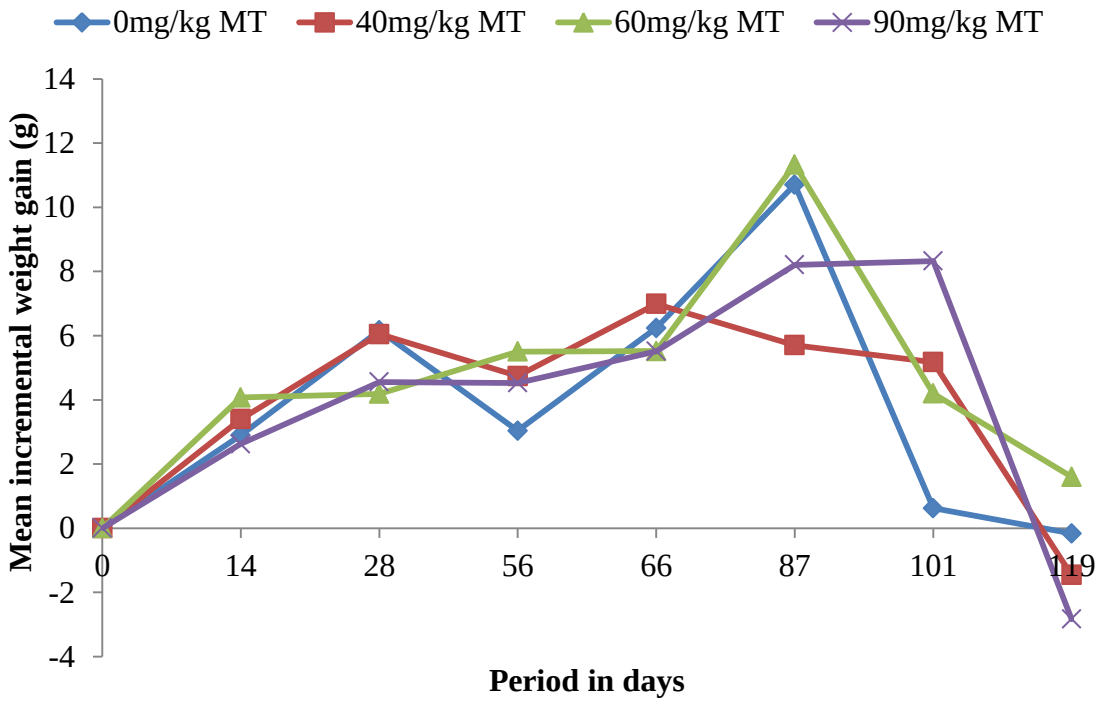


Figure 6.4: Incremental growth trend of *O. andersonii* fed on MT for 119 days

The control group (38.211 ± 1.444g) was similar (P> 0.05) to the fish fed with 40 mgMT/kg MT (38.598 ± 1.180g) and 90 mgMT/kg (39.918 ± 1.414g). The 60 mgMT/kg (44.601 ± 1.414g) fed fish had the highest FMW (g) and this was significantly different (P < 0.05) from the other treatments and the control.

The SGR (%day^{-1}) was not significant (P> 0.05) among the all treatments although the fish fed with 60 mgMT/kg (1.550 ± 0.356%day^{-1}) was the highest followed by the fish

fed with 40 mgMT/kg (1.543 ± 0.462%day^{-1}) and the control group (0 mgMT/kg) (1.423 ± 0.464%day^{-1}). The least SGR (%day^{-1}) was observed in fish fed with the feed incorporated with 90 mgMT/kg.

Weight gain (g) was not significant (P> 0.05) across the treatments although it was highest in the fish fed with 60 mgMT/kg (36.428 ± 1.134g) followed by the 90 mgMT/kg MT fed fish (30.933 ± 1.436g). The least weight gain (29.506 ± 1.424g) was observed in the control group (Table 6.3).

The MWDG (g) followed the similar pattern although the fish fed with 60 mgMT/kg (0.754 ± 0.034g) recorded the highest daily mean weight gain followed by the 40 mgMT/kg fed fish (0.754 ± 0.034g). The least MWDG (g) was observed in *O. andersonii* fed with 90 mgMT/kg incorporated feed (0.668 ± 0.026g) (Table 6.3).

The condition factor (K) was not significant (P> 0.05) across the treatments although the control group recorded the highest (2.139 ± 0.072%) followed by 90 mg/kg MT fed fish (2.089 ± 0.074%), 60 mgMT/kg (2.061 ± 0.053%) and 2.061 ± 0.053% for 40 mgMT/kg fed fish in that order (Table 6.3).

There were no significant differences (P> 0.05) in the CSI (%) among the treatments. However, the highest CSI (%) was observed in 90 mgMT/kg fed fish (0.128 ± 0.020%) followed by the control group (0.115 ± 0.026%), 40 mgMT/kg fed fish (0.096 ± 0.219%) and the least being the *O. andersonii* fed on 60 mgMT/kg (0.087 ± 0.017%) (Table 6.3).

Similarly the HSI (%) did not differ ($P > 0.05$) among the treatments although the control group recorded the highest (0.231±0.028%) (Table 6.3).

Table 6.3: Growth indices of *O. andersonii* fed with different MT levels (mean ± SE)

Treatment	0 mgMT/kg	40 mgMT/kg	60 mgMT/kg	90 mgMT/kg
Final weight (g)	38.211±1.444[a]	38.598 ± 1.180[a]	44.601 ± 1.414[b]	39.918 ± 1.364[a]
SGR (%day^{-1})	1.423±0.464	1.543 ± 0.462	1.550 ± 0.356	1.415 ± 0.362
Weight gain (g)	29.506±1.424	30.604 ± 1.058	36.428 ± 1.134	30.933 ± 1.436
*MDWG (g)	0.704±0.034	0.754 ± 0.034	0.808 ± 0.034	0.668 ± 0.026
K (%)	2.139±0.072	2.061 ± 0.053	2.067 ± 0.063	2.089 ± 0.074
CSI (%)	0.115±0.026	0.096 ± 0.219	0.087 ± 0.017	0.128 ± 0.020
HSI (%)	0.231±0.028	0.147±0.027	0.161±0.029	0.149±0.034

*Mean daily weight gain (MDWG) = weight gain (g)/experimental period. Different superscripts in a row indicate significant difference ($P < 0.05$).

6.4.2 Reproductive performance

Of all the fish investigated 98.75% were mature with the only mature fish was found in 60 mgMT/kg treatment (Table 6.4).

Table 6.4: Reproductive status of *O. andersonii* fed with feed incorporated with different MT levels

	Treatment (mgMT/kg)				
	0	**40**	**60**	**90**	**Total**
Immature	0	0	1	0	1
Mature	20	20	19	20	79
Total	20	20	20	20	80

The relationship between and among MT and reproductive characteristics were investigated using Pearson's product moment correlation coefficient. There was a weak positive correlation between MT level and fish weight ($r = 0.356$, $n = 220$, $P > 0.05$). No relationship was observed between the hormone level and reproductive characteristics of *O. andersonii*. There was a significant ($P < 0.05$) strong positive correlation between fecundity and number of eggs ($r = 0.936$, $n = 220$, $P < 0.05$) and weight of the ovary ($r = 0.706$, $n = 220$, $P < 0.05$) with high fecundity associated with a large number of eggs and ovary weight. Similarly, a significant ($P < 0.05$) strong positive correlation was observed between number of eggs and weight of the ovary ($r = 0.814$, $n = 220$, $P < 0.05$) with high number of eggs associated with heavy ovaries. There was no correlation between number of eggs and weight of fish ($r = 0.05$, $n = 220$, $P > 0.05$). There was a significant ($P < 0.05$) but weak negative relationship between fecundity and weight of fish ($r = -0.270$, $n = 220$, $P < 0.05$) (Table 6.5).

Table 6.5: Pearson Product – Moment Correlation among the reproductive indices

Measures	**1**	**2**	**3**
Fecundity			
Number of eggs	0.936**		
Weight of the ovary	0.814**	0.706**	
Weight of fish	0.137*	0.05	-0.270**

** $P < 0.01$, * $P < 0.05$

The size of eggs was positively correlated to ovary weight (r = 0.417, n = 220, $P < 0.01$) and fecundity (r = 0.32, n = 220, $P < 0.01$). The GSI (%) was not significant ($P > 0.05$) among the treatments although the 40 mgMT/kg had the highest GSI (%) (0.805 ± 0.174%) followed by 90 mgMT/kg fed fish (0.751 ± 0.231%) and 0.738 ± 0.196% for 60 mgMT/kg fed fish. The least GSI (%) existed in fish raised on 60 mgMT/kg feed. The highest GI (%) was observed in 60 mgMT/kg fed fish (36.181 ± 0.883%) with the lowest GI (%) found in the control group although no significant differences ($P > 0.05$) were observed in all the treatments (Table 6.6). There were significant differences ($P < 0.05$) in fecundity between the fish fed with 60 mgMT/kg (500.275 ± 52.318%) and the rest of the treatments including the control group (790.112 ± 40.177%). The 60 mgMT/kg fed fish was significantly lower than the rest of the rest of the treatments. The highest fecundity was recorded in 90 mgMT/kg fed fish (852.304 ± 65.817%) followed by the control group and the fish raised on the 40 mgMT/kg fish (724.368 ± 32.309%) (Table 6.6).

Table 6.6: Reproductive indices of fish fed different levels of MT (mean ± SE)

Treatment	0 mgMT/kg	40 mgMT/kg	60 mgMT/kg	90 mgMT/kg
GSI (%)	0.738±0.196	0.805±0.174	0.512±0.160	0.751±0.231
GI (%)*	33.572±1.166	34.732±1.167	36.181 ± 0.883	34.807±1.067
Fecundity (%)	790.112±40.1 77^{b}	724.368±32.30 9^{b}	500.275±52.31 8^{a}	852.304±65.81 7^{b}
Egg size (mm)	0.922 ± 0.070	0.993 ± 0.049	0.888 ± 0.119	0.871±0.050
Reproductive effort	796.136±79.4 53^{b}	755.146±54.31 1^{b}	484.225±84.07 1^{a}	827.835±98.73 9^{b}

Different superscripts in a row indicate significant difference ($P < 0.05$). *Gonad weight (g)/TL^3(mm).

With the exception of CSI (%) and HSI (%), females had a higher GSI (%) and GI (%) than males and these were significant ($P< 0.05$) (Table 6.7).

Table 6.7: Reproductive indices of fish fed with MT according to sex (mean ± SE)

Treatment	Females	Males
GSI (%)	1.061 ± 0.159^{b}	0.337 ± 0.052^{a}
GI (%)*	32.175 ± 0.597^{a}	37.471 ± 0.677^{b}
CSI (%)	0.110± 0.200	0.111 ± 0.011
HSI (%)	0.166 ± 0.025	0.180 ± 0.019

Different superscripts in a row indicate significant difference ($P < 0.05$).*Gonad weight (g)/TL^3 (mm).

6.4.3 Histological examination

6.4.3.1 Gonads

The representative histological pictures are presented in Figure 6.4. The histological examination of the gonads of both sexes were normal with the ovary having different oocyte stages in all the treatments with MT not having any inhibitory effect on the development of the oocytes. Development of the oocytes was similar in all the replicates in each MT level. The clear oocytes included the perinucleolar, cortical alveolar, vitellogeneic and mature oocytes. Histological sections of the testes in all the treatments showed normal tissue architecture and where reproductively functional as there were spermatozoa and basal membranes.

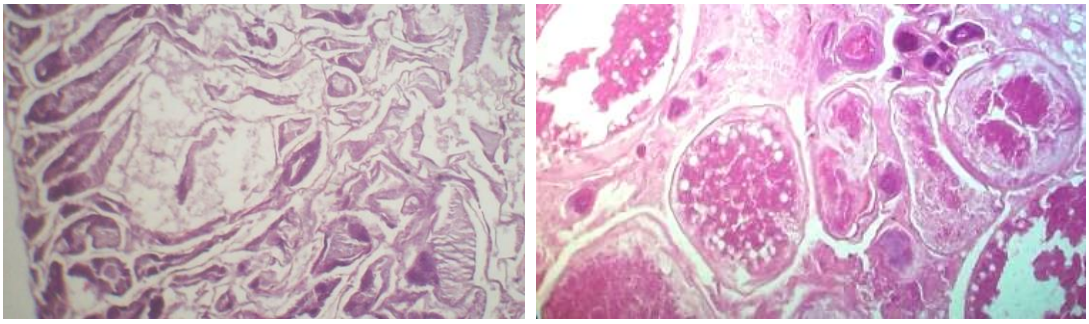


Figure 6.5a: Testis (left) and ovary (0 mgMT/kg treated *O. andersonii*)

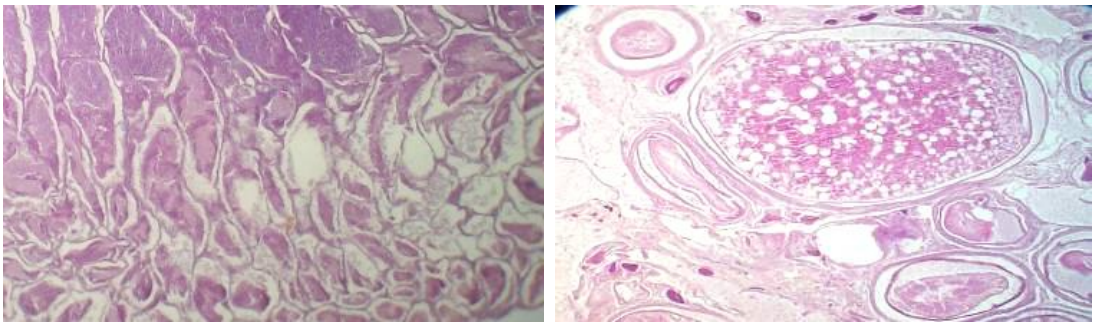


Figure 6.5b: Testis (left) and ovary (40 mgMT/kg treated *O. andersonii*)

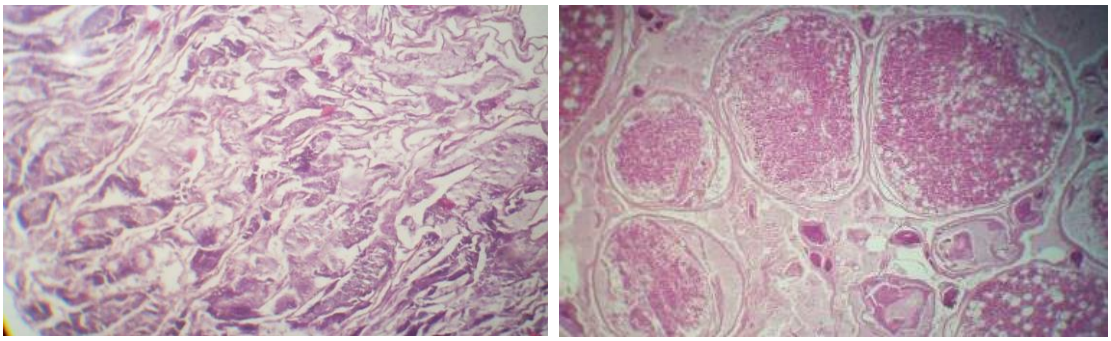


Figure 6.5c: Testis (left) and ovary (60 mgMT/kg treated *O. andersonii*)

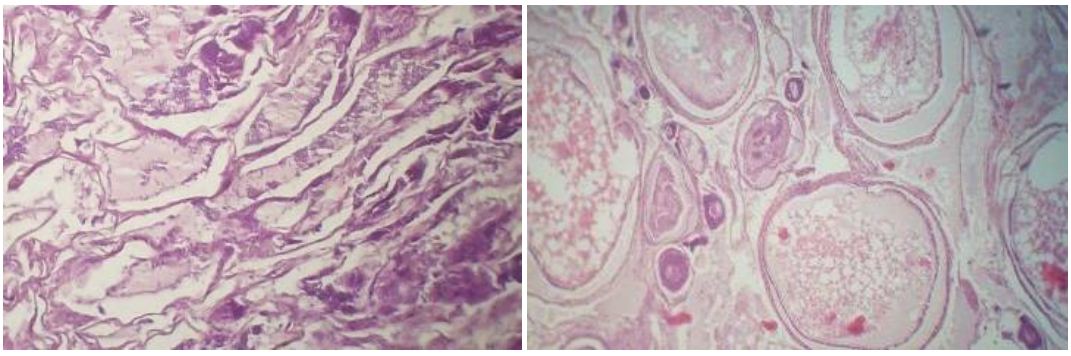
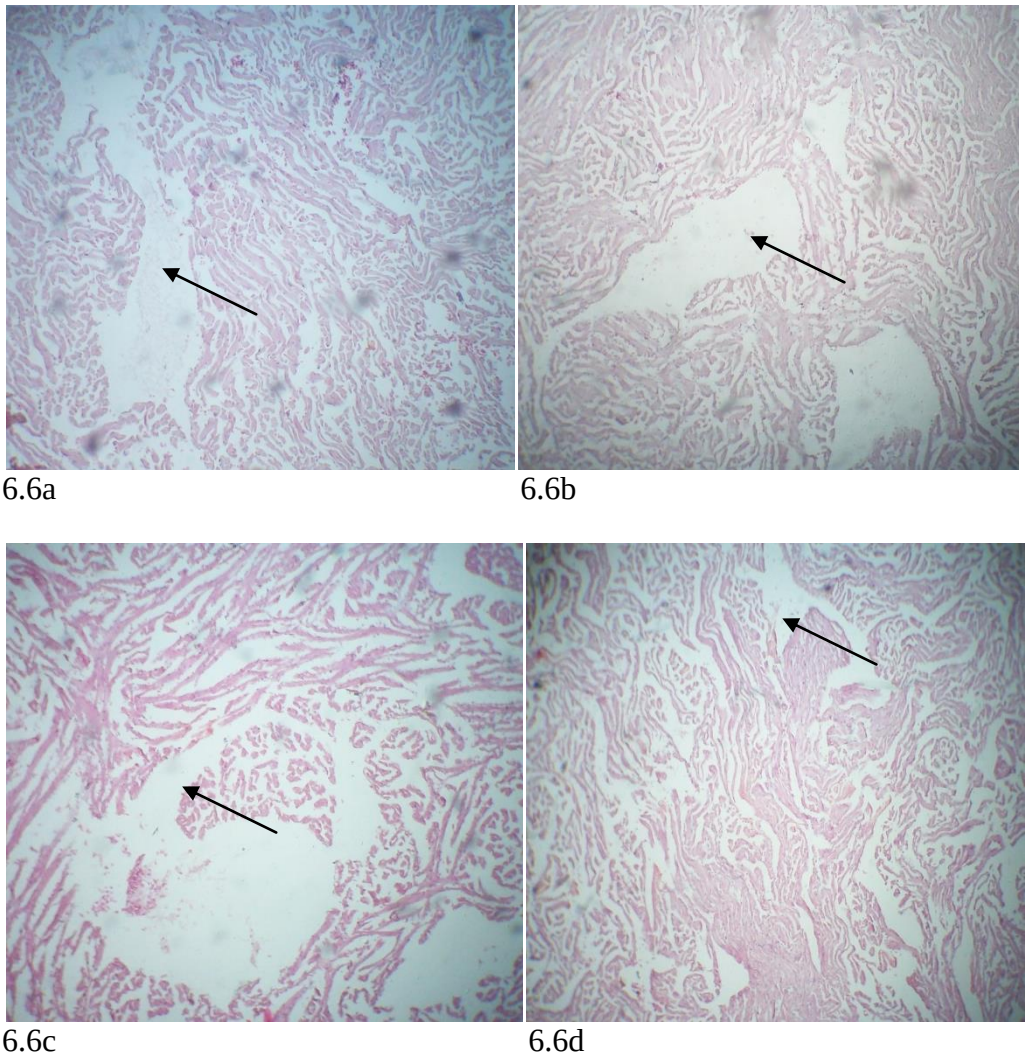


Figure 6.5d: Testis (left) and ovary (90 mgMT/kg treated *O. andersonii*)

6.4.3.2 Heart

Representative histological pictures of the *O. andersonii* hearts fed with the different levels of MT are presented in Figure 6.6.



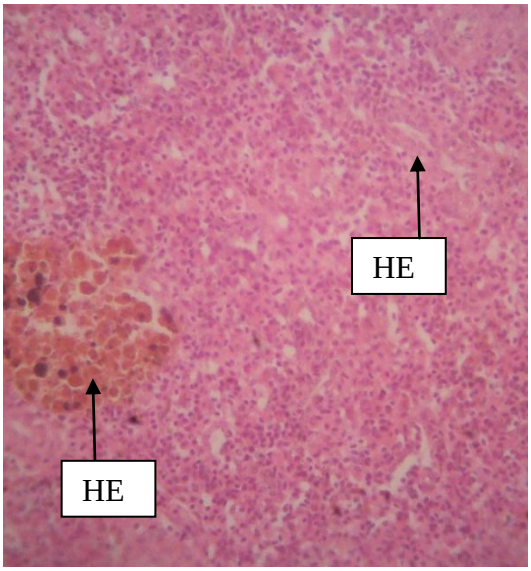
6.6a 6.6b

6.6c 6.6d

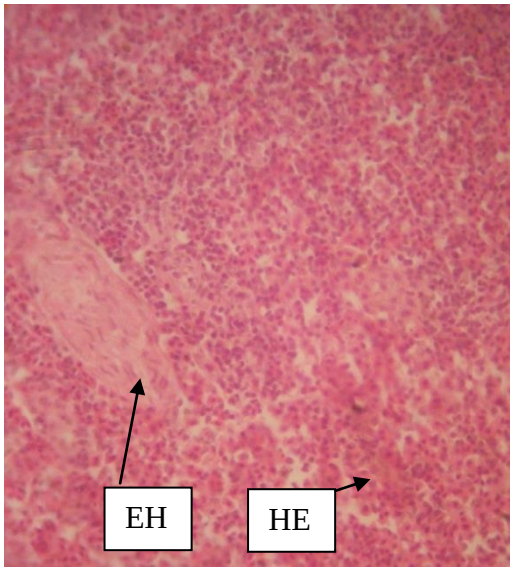
Figure 6.6: Histological pictures of *O. andersonii* heart treated in 0 mg/kg MT (a), 40 mg/kg MT (b), 60 mg/kg MT (c) and 90 mg/kg MT (d) of feed with vacuolar degeneration (arrow).

6.4.3.2 Liver

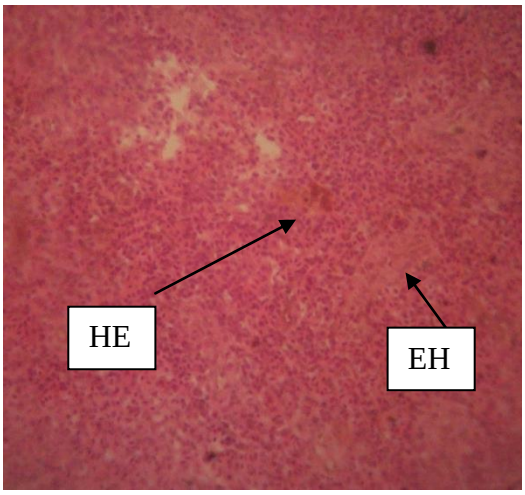
Representative histological pictures of the *O. andersonii* liver fed the different levels MT are presented in Figure 6.7. All the fish exposed to MT showed extended extra-hepatic pancreas and brown granules of haemosidrein (haemochromatosis) in the livers including the control fish.



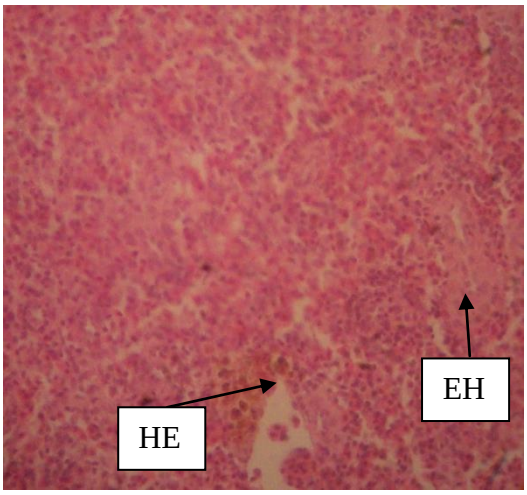
6.7a



6.7b



6.7c



6.7d

Figure 6.7: Histological pictures of *O. andersonii* liver treated in 0 mg/kg MT (a), 40 mg/kg MT (b), 60 mg/kg MT (c) and 90 mg/kg MT (d) of feed showing brown granules of haemosidrein (HE) and extra – hepatic pancreas (EH).

6.4.4 Haematological studies

There were significant differences ($P< 0.05$) observed in RBC, Hb and Ht measured with the control group having significant higher values ($P< 0.05$) than that of the MT treated fish. However, with the exception of PLT, PCT and PDW, the control seemed to have a higher value of the haematological indices than those treated with MT (Table 6.8). Similarly, partial correlation, controlling for fish size between the amount of MT and blood parameters showed negative relationships between MT and haematological parameters with high levels of MT associated with low levels of blood indices. However, only haemoglobin ($r = -0.743$; $P< 0.05$) and RBC ($r = -0.726$; $P< 0.05$) showed significant association with MT levels (Table 6.9).

Table 6.8: Haematological characteristics of *O. andersonii* males subjected to different MT levels (mean ± SE)

Parameter	Statistic	0 mgMT/kg	40 mgMT/kg	60 mgMT/kg	90 mgMT/kg
WBC ($10^3 \mu l^{-1}$)	Mean±SE	145.6±10.830	80.700±12.890	61.867±24.618	94.567±37.582
	Minimum	125.6	55.0	15.3	30.9
	Maximum	162.8	95.3	99.0	161.0
• LYM ($10^3 \mu l^{-1}$)	Mean±SE	142.867±10.038	79.00±12.864	60.6±24.23	90.033±34.209
	Minimum	124.2	53.3	14.7	30.5
	Maximum	158.6	92.9	96.9	149.0
• MON ($10^3 \mu l^{-1}$)	Mean±SE	1.667±0.521	0.967±0.348	0.7±0.321	1.633±1.058
	Minimum	0.8	0.4	0.2	0.2
	Maximum	2.6	1.6	1.3	3.7
• GRA ($10^3 \mu l^{-1}$)	Mean±SE	1.067±0.291	0.733±0.067	0.567±0.12	0.9±0.7
	Minimum	0.1	0.6	0.2	0.2
	Maximum	1.6	0.8	0.8	2.3
• LYM (%)	Mean±SE	98.2±0.436	97.867±0.649	97.6±0.625	96.733±2.067
	Minimum	97.4	96.90	96.4	92.6
	Maximum	98.9	99.1	98.5	98.9
• MON (%)	Mean±SE	1.167±0.291	1.367±0.384	1.400±0.289	2.167±1.272
	Minimum	0.7	0.6	0.9	0.70
	Maximum	1.70	1.80	1.90	4.70
• GRA (%)	Mean±SE	0.633±0.145	0.767±0.291	1.0±0.352	1.1±0.802
	Minimum	0.4	0.3	0.6	0.2
	Maximum	0.9	1.3	1.7	2.7
RBC ($10^6 \mu l^{-1}$)	Mean±SE	1.307 ± 0.344^b	0.747 ± 0.124^a	0.627 ± 0.145^a	0.763 ± 0.187^a
	Minimum	0.62	0.53	0.34	0.49
	Maximum	1.7	0.96	0.81	1.12
Hb (g/dL)	Mean±SE	7.067 ± 0.696^b	4.333 ± 0.633^a	3.567 ± 0.524^a	5.033 ± 1.071^a
	Minimum	5.8	3.1	2.6	3.1
	Maximum	8.2	5.2	4.4	6.8
Ht (%)	Mean±SE	15.633 ± 5.005^b	7.067 ± 1.23^a	8.033 ± 2.805^a	7.867 ± 0.606^a
	Minimum	8.3	5.1	2.8	6.8
	Maximum	25.2	9.3	12.4	8.9

Table 6.8 Continued

Parameter	Statistics	0 mgMT/kg	40 mgMT/kg	60 mgMT/kg	90 mgMT/kg
MCV (μm^3)	Mean±SE	299.333±16.667	169.0±60.008	121.667±25.221	227.333±45.038
	Minimum	266.0	96.0	85.0	138.0
	Maximum	316.0	288.0	170.0	282.0
MCH (pg)	Mean±SE	94.133±2.083	77.433±17.013	60.9667±8.207	85.567±11.712
	Minimum	90.4	58.70	51.4	62.7
	Maximum	97.60	111.4	77.3	101.4
MCHC (p/dL)	Mean±SE	63.267±3.994	63.100±7.835	56.867±17.982	62.700±9.322
	Minimum	57.2	50.4	30.2	45.3
	Maximum	70.8	77.4	91.1	77.2
RBCDW (%)	Mean±SE	29.3±8.55	27.933±6.233	25.167±4.446	11.9±0.902
	Minimum	14.8	16.4	17.4	10.9
	Maximum	44.4	37.8	32.8	13.7
PLT ($10^3 \mu l^{-1}$)	Mean±SE	64.0±21.166	50.058±25.267	92.667±19.402	35.0±13.0
	Minimum	32.0	174.0	56.0	22.0
	Maximum	104.0	82.0	122.0	48.0
MPV(μm^3)	Mean±SE	12.000±1.000	11.500±1.735	6.5±0.361	9.5±3.1
	Minimum	11.0	8.1	6.0	6.4
	Maximum	164.0	13.8	7.2	12.6
PCT (%)	Mean±SE	0.032±0.007	0.101±0.060	0.061±0.014	0.015±0.001
	Minimum	0.018	0.028	0.033	0.014
	Maximum	0.048	0.219	0.077	0.015
PDW (%)	Mean±SE	9.3±3.073	12.3±3.623	6.2+0.635	7.0±0.1
	Minimum	5.6	5.2	5.1	6.9
	Maximum	15.4	17.1	7.3	7.1

Different superscripts in a row indicate significant difference ($P < 0.05$).

Table 6.9: Partial correlation of haematological activities of *O. andersonii* with MT levels

	WBC (mm^3)	**RBC (mm^3)**	**Hb (g/dL)**	**Ht (%)**	**MCV (um^3)**	**MCH (pg)**	**MCHC (p/dL)**	**RBCDW (%)**	**PLT (mm^3)**	**MPV (um^3)**	**PCT (%)**	**PDW (%)**
(r)	-0.693	-0.726	-0.743	-0.461	-0.669	-0.606	-0.196	-0.434	-0.006	-0.360	-0.049	-0.318
(P)	0.057	0.041	0.035	0.250	0.069	0.111	0.642	0.282	0.988	0.382	0.907	0.442

Table 6.10: Plasma chemistry for *O. andersonii* subjected to different MT levels (mean ± SE)

Parameter	Statistic	0 mgMT/kg	40 mgMT/kg	60 mgMT/kg	90 mgMT/kg
Uric acid ($\mu molL^{-1}$)	Mean±SE	45.200±7.211	33.900±4.590	17.725±2.130	44.683±22.733
	Minimum	25.500	20.70	6.90	9.10
	Maximum	62.800	49.10	16.10	152.50
Urea ($mmolL^{-1}$)	Mean±SE	0.638 ± 0.047^{a}	0.923 ± 0.042^{b}	0.617 ± 0.060^{a}	0.617 ± 0.042^{a}
	Minimum	0.530	0.720	0.510	0.470
	Maximum	0.820	1.040	0.760	0.690
Creatinine ($\mu molL^{-1}$)	Mean±SE	19.750±3.977	29.333±3.558	27.333±5.031	22.833±3.558
	Minimum	0.000	22.000	23.000	19.000
	Maximum	32.000	53.000	30.000	30.000
Albumin (gL^{-1})	Mean±SE	9.975±0.484	10.133±0.433	10.150±0.613	9.650±0.433
	Minimum	8.600	8.500	9.900	8.600
	Maximum	11.400	12.200	16.400	10.900
Total Bilirubin ($\mu molL^{-1}$)	Mean±SE	-1.867±1.618	-5.083±1.447	-2.733±2.046	-1.083±1.447
	Minimum	-6.10	-12.600	-7.700	-2.200
	Maximum	-0.60	-1.000	-0.400	0.100
LDH (μL^{-1})	Mean±SE	449.833±82.549	561.167±73.834	433.000±104.417	379.667±73.834
	Minimum	178.000	311.000	299.000	218.000
	Maximum	881.000	840.000	534.000	681
Total protein (gL^{-1})	Mean±SE	26.050±1.399	26.350±1.251	30.317±1.770	27.733±1.251
	Minimum	21.900	21.000	27.700	26.100
	Maximum	31.500	33.1000	31.900	31.200
Cholesterol ($mmolL^{-1}$)	Mean±SE	3.225±0.359	3.283±0.321	3.383±0.454	3.100±0.321
	Minimum	1.900	1.700	2.400	2.500
	Maximum	4.600	5.100	3.900	4.600
Triglycerides ($mmolL^{-1}$)	Mean±SE	0.993±0.182	0.790±0.163	0.778±0.230	0.807±0.163
	Minimum	0.450	0.410	0.460	0.420
	Maximum	1.550	1.380	1.080	1.200
Globulins (gL^{-1}))	Mean±SE	16.160±1.262	16.217±1.383	19.300±1.023	18.083±0.531
	Minimum	12.300	10.800	16.900	16.600
	Maximum	20.100	20.900	21.900	20.400

Different superscripts in a row indicate significant difference ($P < 0.05$).

6.4.5 Cost analysis

There was a strong positive correlation between total cost of the feed used and the MT level (r = 0.747, n = 56, $P< 0.05$) with an increased level of MT associated with an increased total cost. Significant differences ($P< 0.05$) were observed in the cost of the feed with the 90 mgMT/kg (ZK4, 268.37 ± 589.92) recording the highest feed cost. The 40 mgMT/kg and 60 mgMT/kg feed costs were not significant different ($P> 0.05$) although the latter (ZK2, 973.17 ± 432.11) had the higher feed cost than the former (ZK1, 926.01 ± 260.11).

Total revenue was not significant ($P> 0.05$) across the treatments although the 60mg/kg MT (ZK17, 537.02 ± 2, 045.53) gave the highest income followed by the 40 mgMT/kg (ZK16, 617.78 ± 1, 766.67).The lowest TR was realized from the fish fed with the 90 mgMT/kg (ZK16, 445.11 ± 1, 867.72) (Table 6.11).

The GM declined (r = -0.25, n = 56, $P > 0.05$) with the level of MT incorporation in the feed although no significant differences ($P > 0.05$) were observed across the treatments. The control (0 mgMT/kg) had the highest GM (ZK16, 417.36 ± 1, 732.76) followed by the 40 mgMT/kg (ZK14, 691.77 ± 1, 517.31), 60 mgMT/kg (ZK14, 563.85 ± 1, 642.05) and 90 mgMT/kg (ZK12, 176.74 ± 1, 322.39) in that order. The gross margin ratio was significant ($P< 0.05$) across the treatments with the control group recording the highest ratio (1.000), followed by the 40 mgMT/kg fed fish. The least ratio was observed in fish subjected to the 90 mgMT/kg treatment (Table 6.11).

Table 6.11: Cost analysis of the of the MT inclusion in the feed fed to *O. andersonii* for 119 days (mean ± SE)

Treatment (mg/kg)	**0 mgMT/kg**	**40 mgMT/kg**	**60 mgMT/kg**	**90 mgMT/kg**
Total cost (ZK)	0	1, 926.01 ± 260.11[a]	2, 973.17 ± 432.11[a]	4, 268.37 ± 589.92[b]
Total Revenue (ZK)	16, 417.36 ± 1, 732.76	16, 617.78 ± 1, 766.67	17, 537.02 ± 2, 045.53	16, 445.11 ± 1, 867.72
Gross margin (ZK)	16, 417.36 ± 1, 732.76	14, 691.77 ± 1, 517.31	14, 563.85 ± 1, 642.05	12, 176.74 ± 1, 322.39
Gross margin ratio	1.000 ± 0.000[d]	0.886 ± 0.005[c]	0.837 ± 0.006[b]	0.747 ± 0.010[a]

Different superscripts in a row indicate significant difference ($P < 0.05$).

Table 6.12: Water quality parameters in the MT experiment subjected to *O. andersonii* for 119 days (mean ± SE**)**

Treatment	**0 mgMT/kg**	**40 mgMT/kg**	**60 mgMT/kg**	**90 mgMT/kg**
Water temperature (°C)	23.656 ± 0.205	23.638 ± 0.212	23.645 ± 0.208	23.645 ± 0.208
pH	6.95 ± 0.08	7.03 ± 0.06	7.08 ± 0.07	6.98 ± 0.08
Conductivity (µS/cm)	0.096 ± 0.006	0.089 ± 0.007	0.096 ± 0.006	0.095 ± 0.006
Nitrite (mg/L)	0.114 ± 0.032	0.118 ± 0.034	0.198 ± 0.114	0.193 ± 0.115
DO (mg/L)	7.042±0.364	7.024±0.297	7.606±0.456	7.274±0.390

6.5 Discussion

Oreochromis andersonii is an important fish species in Zambian aquaculture despite few publications in the public domain about this fish species. There is no reported use of MT as an ingredient in the production of fish feed meant for grow – out. Similarly there are no reports of using the hormone in the brood stock feed. This could be attributed to the non-supportive evidence of its positive influence on both growth and reproductive traits of major biological and economic importance in other fish species. In this study, the final weight of 60 mgMT/kg fed fish was the highest among the other treatments (40 mgMT/kg and 90 mgMT/kg) including the control (0 mgMT/kg). However, the SGR (% day^{-1}), MDWG and weight gain were not significant different (P> 0.05). Organ indices (HSI and K) were not significant (P> 0.05) too.

Anabolic steroids, both androgens and estrogens, enhance growth (Matty, 1985; Hossain *et al*., 2002). In this study, the FMW of fish fed with 60 mgMT/kg was higher than the control group. However, the control group was not different from the 40 mgMT/kg and 90 mgMT/kg. The results are consistent with what Vatanakul *et al*. (1993) found when they studied on grouper (*Epinephelus malabaricus*). In their study they found that the control (0 mgMT/kg) and 60 mgMT/kg gave similar results but were higher than the 40, 80 and 100 mgMT/kg. Marjan *et al*. (2009) found that 75 mgMT/kg gave the highest body weight gain which was 1.3 times higher than the control when they studied on *O. mossambicus.* Mateen (2007) found 70 mgMT/kg to have the highest positive effect on the growth indices of *O. niloticus*. The gain in body weight was also higher than at the

50 and 100 mgMT/kg dose. In the current study, the highest dosage showed similar results to 40 mgMT/kg and the control group.

In the sex reversal of Tilapia fish species several dosages of MT have been used and recommended (50 mg/kg (Owuso – Frimpong and Nijjar, 1981); 40 mg/kg (Abucay and Mair, 1997); 60 mg/kg (Tave, 1990; Romerio *et al*., 2000); 75 mg/kg (Marjani *et al*., 2009). Higher doses have been reported to be deleterious and catabolic and therefore reduce growth instead of being anabolic in nature. Jae – Yoon *et al*. (1988) reported a depression in fish growth when the dose of MT was increased from 60 mgMT/kg to 80 mgMT/kg of feed. The MT rate at which growth was maximized coincides with the 60 mgMT/kg that has been recommended by several authors (Tave, 1990; Bhujel, 2011) on *O. niloticus* in all male production, the species closest to the fish under study. Therefore, the lower growth exhibited by the 0 and 40 mgMT/kg may be as a result of having little anabolic effect probably due to its low dosage while the 90 mgMT/kg might have had catabolic effects and therefore low FMW of the fish (*O. andersonii*) under study.

Reproduction in teleost fish is well known to be controlled by the environmental cues (Crim, 1982; Lam, 1983; Stacey, 1984; Lee *et al*., 1986). This is because the cues mediate secretion of hormones by the brain and pituitary to synchronize the activities of various organs involved in reproduction. Lokman *et al*. (2007) showed that 11 – KT can exert direct effects on the eel ovary resulting in the increases in size of previtellogenic oocytes. Similarly, Kortner and Arukwe (2008) implicated 11 – KT and testosterone in inducing the growth and development of previtellogenic oocytes in Cod (*Gadus morhua*).

The effect of MT on gonads appears to be complex (Ahmed *et al*., 2002). In the current experiment, there was no evidence of MT having an effect on the reproductive characteristics of *O. andersonii* since the GSI, GI and egg size were not significantly different ($P > 0.05$). This was also confirmed by the functional gonads as observed by the histological examination of the gonads although they were at different maturity stages. However, fecundity was significant ($P < 0.05$) with 60 mgMT/kg fed fish having the lowest fecundity. This could be explained by the fact that the fish reared on 60 mgMT/kg had the highest FMW since fecundity is calculated by dividing the number of eggs over the fish body weight. This is supported by the correlation analysis that showed a weak positive relationship ($r = 0.137$, $n = 220$, $P < 0.05$) between the fish weight and ovary weight although fecundity was positively correlated ($r = 0.706$, $n = 220$, $P < 0.05$) to ovary weight. However, low fecundity shows that fish fed with 60 mgMT/kg used fewer resources in the egg production. This is confirmed by the reproductive effort which was the lowest among the treatments.

Reproductive activity demands a lot of energy leading to physiological trade – off in available energy resulting in reduced growth (Aday, 2002). In the current experiment, the 60 mgMT/kg fed fish had little reproductive effort, therefore, resulting in increased growth as evidenced by the significant ($P < 0.05$) highest FMW. This is in response to environmental conditions since the adaptability and plasticity of tilapias are well known (Fryer and Iles, 1972). In unstable environment a more r – selected strategy is characterized by high fecundities, fluctuating density – independent mortalities, fast generation overturn and more generalized feeding (Ojuok *et al*., 2007). High

reproductive effort signifies stressful environment. In the current study, the 60 mgMT/kg might have positive effect on fish growth resulting in low reproductive effort. However, other environmental cues apart from MT may be at play since other reproductive characteristics were not significant ($P > 0.05$) across the treatments. Lokman *et al*. (2007) used an *in vitro* method while in the current study the fish were cultured in the outdoor tanks similar to culture conditions. In addition, the two experiments used different fish species. These may be reasons as to why results are different.

In all the fish subjected to different MT levels, there was occurrence of varying eggs indicating development of eggs at different times and that signifies multiple spawning by the *O. andersonii*. In this experiment the egg size ranged between 0.1 – 3.40mm. The maximum egg size is less than the 2.1 – 3.7mm reported by Peña – Mendoza *et al*. (2005) in *O. niloticus*. Similar observations were made on *O. andersonii* by Kefi *et al*. (2012).

The values for total protein, globulins, albumin and cholesterol are within the ranges reported in Tilapia hybrid by Hrubec *et al.* (2000). Although the value of the cholesterol in the current experiment was lower than that reported by Chen *et al*. (2003) in healthy *O. niloticus* globulin and albumin were similar ($P > 0.05$). Total protein was within the ranges of *O. niloticus, O. aureus* and the hybrid between *O. niloticus* and *O. aureus* although uric acid and creatinine were higher than the values obtained by El – Hawarry (2011). However, there were no significant differences ($P > 0.05$) among the treatments indicating that the liver protein metabolism and kidney function were similar in MT treated and the control *O. andersonii*. This was also confirmed by the histological

analysis of liver which did not show any pathological differences among the treatments. Deborah (1990) did not find any deviation in the morphology of the liver between the untreated and treated channel catfish *Ictalurus punctatus* with MT.

Total bilirubin in the current study showed negative values indicating very low levels in the blood. This could be attributed to haemolysis (which is the rapturing of the RBC and the release of cytoplasm into the sorounding fluid which is blood blood plasma) of the blood during analysis and therefore cannot be compared to any other studies. However, no significant differences ($P > 0.05$) were shown among the treatments meaning that comparison can only conducted among the treatments in the current experiment.

High dosages of exogenous male hormones are known to cause side effects, especially liver damage. However, lower levels have been established to offer various health benefits, including reduced risks from cardio-vascular disease and cancer (Hasheesh *et al*., 2011). If male hormones are administered to humans within physiological ranges, their side effects are kept to the minimum (Bhasin *et al.*, 1998; Hasheesh *et al*., 2011). Hasheesh *et al*. (2011) did not observe any significant differences ($P > 0.05$) in the physiological parameters (plasma total protein, albumin, globulin, A/G ratio, total lipids, cholesterol, aspartate amino transferase (AST), alanine amino transferase (ALT) and LDH) between untreated and treated *O. niloticus* with MT at 30 and 60 mgMT/kg. This is consistent to the results of the current experiment that did not reveal any differences ($P > 0.05$) among the treatments in the blood chemistry parameters.

Several methods have been employed in assessing the condition of the fish. However, haematological parameters have been described to be reliable (Katalog and Parlak, 2004; Akinrotimi *et al*., 2012) as they indicate the nutritional status and overall health indication of the fish (Akinrotimi *et al*., 2012). Although the control fish had higher values of WBC, MCV, MPV, MCHC and RBCDW than the treatments, there were no significant differences ($P> 0.05$) between the control and the treatments and among the treatments. The control fish had higher RBC than the MT fed fish and this was significant ($P< 0.05$). However, RBC and WBC were within the reference ranges provided by Hrubec *et al*. (2000) for *O. niloticus* but lower than those found by Ighwela *et al*. (2012). However, both Hb and Ht were lower than those found by Adedeji and Adegbile (2011) in Bagrid catfish (*Chrysichthys nigrodigitatus*) and African catfish (*C. gariepinus*). The control fish had significantly higher ($P< 0.05$) RBC, Hb and Ht and within the reference ranges by Hrubec *et al*. (2000). The decrease in Ht and Hb by the fish given MT may signal condition deterioration as a result of androgen administration. The high number of WBC and lymphocytes in the control group may indicate adequate immune responses since their amount has an implication in immune responses and the ability of fish to fight infection (Douglas and Jane, 2012; Adedeji and Adegbile, 2011).

The histopathogy of fish heart has not received much attention in aquaculture research and scanty research is available. There were alteration (vacuolar degeneration) of the fish heart in all the treatments and the control and this histopathological status were similar. Therefore, the changes can not be attributed to MT but probably to other factors. The

changes in the fish heart were observed by Magar and Dube (2013) on the heart of *Channa punctus* exposed to Malathion.

The liver plays a key role in the metabolism and biochemical transformations of pollutants from the environment, which inevitably reflects on its integrity by creating lesions and other histopathological alterations of the liver parenchyma or the bile duct (Velkova – Jordanoska and Kostoski, 2005). There were no histopathological differences observed in the livers between the control and MT treated fish. However, brown granules of haemosidrein (HE) and extra – hepatic pancreas were observed in the liver tissue. Similar observation were made by Abd – Algadir *et al*. (2011) in the liver tissue of *O. niloticus* captured from water suspected to have been polluted by a herbicide Pendimethalin. The former indicate the destruction of blood and is a step in the disintegration of haemoglobin and the formation of bile pigment. Haemosidrein has higher presence of iron and is considered as an iron overload disorder. It is speculated that the presence of brown granules was caused by haemorrhage due to the inflammation of tissue and cannot be attributed to the MT oral administration. This is because the rapid and continued destruction of RBC and breakdown of haemoglobin may have converted them into hemosiderin (Yacoub and Abdel Satar, 2003). All the samples exhibited extra – hepatic pancreas a classical content of glycogen, which fills most of the cytoplasm. Extra – hepatic pancreas develops around the portal vein during ontogenesis.

The study shows that the oral administration of MT for up to 30 days does not affect the physiology of *O. andersonii* as the haematology chemistry and histopathology of the liver

and heart were similar to that of the control. The safety of MT to *O. andersonii* is, therefore, proved under the condition of the current experiment similar to the adopted sex reversal practices. However, they are signs of condition deterioration as manifested in the anaemic conditions in high levels of MT.

The total cost of MT inclusion in the feed increased ($r = 0.747$, $n = 56$, $P< 0.05$) with level of MT. However, there is no evidence to show GM declined with MT level (($r = -0.250$, $n = 56$, $P> 0.05$). The highest GM was found when feed was not incorporated with MT. The total revenue, however, was highest with the fish fed with feed incorporated with 60 mgMT/kg. This could be as a result of the high SGR ($\%day^{-1}$) resulting in the highest FMW shown in the same treatment.

Gross margin ratio is the ratio of gross profit of a business to its revenue. It is a profitability ratio measuring what proportion of revenue is converted into gross profit (i.e. revenue less cost of goods sold). Higher values indicate that more money is earned per unit amount of revenue which is favourable because more profit will be available to cover non – production costs. In the current study, the gross margin ratio was of each treatment was significant ($P< 0.05$). The highest was on the control fish group (1.000 ± 0.000), followed by 40 mgMT/kg fed fish (0.886 ± 0.005) and 60 mgMT/kg fed fish (0.837 ± 0.006). The least gross margin ratio was calculated in the 90 mgMT/kg fed fish (0.747 ± 0.010). This shows that the control group gave more money for the revenue collected.

While the final body weight was highest among the treatments with the feed incorporated with the 60 mgMT/kg the highest GM was observed in the control group (without MT). This shows that biologically the 60 mgMT/kg can be incorporated in the feeding programme to increase the final body weight. However, non – incorporation of the synthetic androgen in the feed is economical since the GM was the highest and therefore incorporation of MT in the feed would just increase unnecessary production costs.

CHAPTER 7

GROWTH AND SEX RATIOS IN 17 α - METHYL TESTOSTERONE TREATED *OREOCHROMIS ANDERSONII*

7.1 Introduction

Culture of monosex *Oreochromis* species, preferably males, has been recognized as the most effective way of avoiding early maturation and uncontrolled reproduction. This is because Tilapia fish exhibits sexually related dimorphic growth in which males grow and reach a larger ultimate size faster than the females (Guerrro, 1975; Mair and Little, 1991; Manosroi *et al.*, 2004). This can be achieved through manual sexing, hormonal administration, chromosome manipulation and hybridization. Of these, hormonal administration has been found to be easy and most effective (Green and Teichert – Coddington, 2000; Mateen, 2007). The method involves the use of synthetic or natural steroidal hormone administered to sexually undifferentiated fish at a given dosage and duration. These sex hormones modify secondary sex characteristics and gonads (Yamamoto, 1951).

However, the use of hormones has environmental risks if not properly managed. In addition, the use of these hormones for food animal production is not allowed in some countries (Baroiller and Toguyeni, 1995). Some hormones such as MT can be harmful by inhalation, ingestion or skin absorption and may cause irritation (Manosroi *et al*., 2004).

Several androgenic hormones (methyl testosterone, ethinyl testosterone, mibolerone, methyl dihydrotestosterone, hydroxyandrostenedione, dihydrotestostorone, testosterone acetate, testosterone propionate and 11 – KT) have been used in the masculinisation of the fish. Of these androgens, the synthetic MT has been widely used (Green and Teichert – Coddington, 2000; Khalil *et al.*, 2011) probably due to its simplicity and reliability.

Although, feed containing MT at 60 mgMT/kg feed dose is used generally for the newly-hatched individuals in the sex reversal studies with oral administration (Pompa and Green, 1990), there are various different doses reported. Guerrero (1975) recommended 30 mgMT/kg while Bhandari *et al*. (2006) found 50 mgMT/kg feed as the most effective dose in masculinisation of the *O. niloticus*. Macintosh and Little (1995) reported 30 – 60 mgMT/kg feed as the general dose used in Nile tilapia juveniles until the 25 – 60th days of early feeding. The differences would be due to variances in the experimental conditions.

Although there have been studies (Green and Teichert – Coddington, 2000; Celik *et al*., 2011) on the effectiveness of the MT on masculinisation of the *O. niloticus*, there has been no published information on *O. andersonii*. In addition, research has concentrated on laboratory experiments whose results applications have produced varying sex reversal success. This study observes the effect of different doses of MT (0, 40, 60 and 90 mgMT/kg feed) on the sex reversal of *O. andersonii*, an important fish species in the Zambian aquaculture.

7.2 Materials and method

Oreochromis andersonii breeders were stocked in 250m^2 semi – concrete pond at the sex ratio of 3:1 (female: male) (Table 7.1). Before stocking the fish, pond was prepared as described in section 3.1.

Table 7.1: Weight of fish (g) stocked for collection of eggs (mean ± SD)

Sex	No. of fish	Weight of fish (g)	SL (mm)	TL (mm)
Males	7	174.857 ± 19.828	165.429 ± 10.406	132.786 ± 21.441
Females	21	71.191 ± 12.093	121.905 ± 9.534	170.536 ± 24.979

After twenty one (21) days, the eggs were recovered from the mouth of the brooding female fish and were immediately taken to the indoor hatchery for incubation in the jars for 48 hours (Figure 7.1). The temperature was set at 28°C with the help of the thermo controlled heater panel immersed in the overhead and bottom tanks. Upon exhaustion of the yolk, the fry were taken to the four concrete tanks (10 x 5 x 0.7m) as described in section 6.2.2.

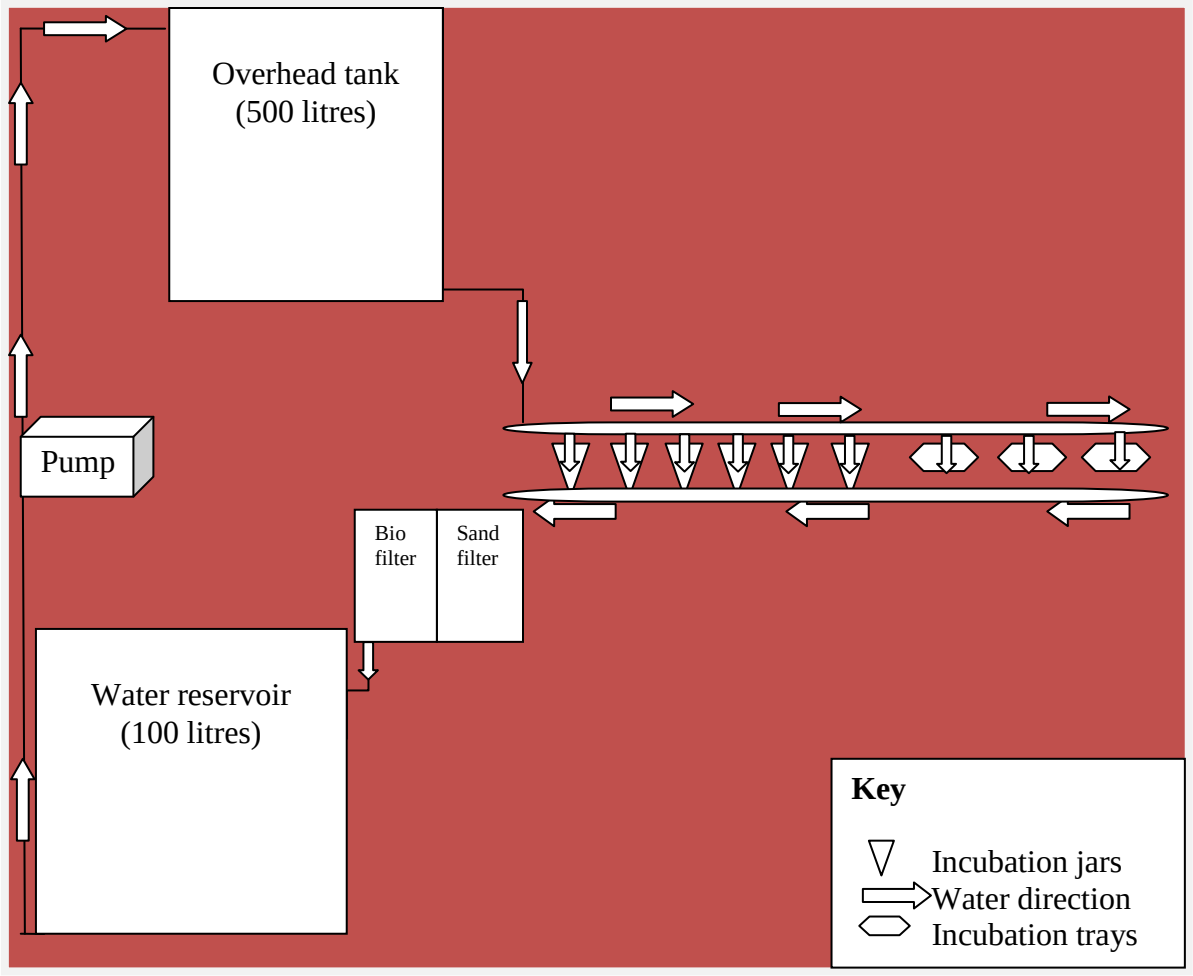


Figure 7.1: Design of an indoor hatchery where the eggs of *O. andersonii* were incubated

7.2.1 Preparation of the concrete tanks and hapas

Four outdoor concrete tanks (10 x 5 x 0.7m) were drained, cleaned and allowed to dry for three days. Four hapas (1 x 1 x 1m) were sown and fixed into four concrete tanks (one hapa in each tank) (Figure 7.2). Water was then pumped into the tanks up to the depth of 40cm leaving 30cm freeboard to avoid the spilling of water since the water was receiving the feed containing with the hormone. In addition, the outlet pipes were sealed with plastics.

The hatched spawn were then taken to the set hapas. In order to determine the mean weight of the spawn, 100 fry were put in a beaker (filled with water) with the known weight. The mean weight was calculated as follows:

$$\text{Mean weight of fry (g)} = \frac{\text{Weight of the beaker with fry (g)} - \text{weight of beaker (g)}}{100}$$

Figure 7.2: Hapas set in the concrete tanks ready for sex reversal experiment

The mean weight of fry was found to be 0.04g. A total of 500 fry were stocked in each hapa (500 fry/m^2) with treatments allocated randomly.

7.2.2 Preparation of the feed and administration of the hormone

Isonitrogenous (30%) and isocaloric (4.02 kcal/g) diet supplemented with three levels of MT was prepared and stored as described in section 6.2.3. The fry were provided with feed at 10% body weight four times a day for a period of 28 days.

7.2.3 Growth monitoring of sex reversed and non sex reversed fish

The juvenile fish were scooped from the hapas and were transferred into the hapas set in the 750m^2 semi – concrete pond. Before stoking, the pond was prepared by draining the water and applying lime (0.1kg/m^2). Twelve hapas were fixed as described in section 4.2.3 since each treatment was replicated thrice. By the 28th day, significant differences ($P< 0.05$) were observed in the weight and length of the fish with the 40 mgMT/kg feed recording the highest mean weight and length (SL (mm) and TL (mm)) (Table 7.2).

Table 7.2: Weight and length of fish stocked in the hapas (mean ± SE)

Treatment	0 mgMT/kg	40 mgMT/kg	60 mgMT/kg	90 mgMT/kg
Mean weight (g)	0.254±0.014^a	0.470±0.019^c	0.384±0.032^b	0.392±0.024^b
SL (mm)	15.960±0.244^a	20.260±0.217^c	19.180±0.374^b	18.880±0.374^b
TL (mm)	20.680±0.324^a	26.280±0.290^c	23.800±0.517^b	24.720±0.470^b

Different superscripts in a row indicate significant difference ($P < 0.05$)

The fish were provided with the 20CL30CP formulated as described in chapter 3 fed at 5% live body weight adjusted monthly after sampling. Mortality was observed and recorded, if any, daily. Sampling was done monthly by taking the weights and lengths (SL and TL) of 25 fish from each hapa or 75 fish from every treatment according to Skelton (2001).

7.2.4 Water quality parameters

Water temperature and pH were determined using the mercury thermometer and pH meter probe, respectively.

7.2.5 Determination of sex in *O. andersonii*

After three months of rearing, 100 fish from each treatment and control were scooped from the hapas, killed and preserved in 10% formalin. The preserved fish were held for 10 days before they were dissected for the evaluation of treatment efficacy. Before dissection, the fish were sexed by checking the genital papillae with the help of the magnifying glass by an independent person from the gonadal examiner. The fish were cut near the anus to the base of the pectoral fin. The gonad was then recovered from the dorsal portion of the peritoneal lining. A gonadal examination technique was carried as described by Guerrero and Shelton (1974) on a Nikon profile projector (V – 12) (10 x power) (Figure 7.3). The presence of previtellogenic or vitellogenic oocytes and the lobular configuration revealed the ovary and testis, respectively and consequently the phenotypic sex (Baroiller *et al.*, 1996) or intersex (gonads containing both ovarian and testicular tissues) (Marjani *et al.*, 2009).

Figure 7.3: Nikon profile projector (V – 12) used for identification of the fish sex

7.3 Data analysis

Data analysis was conducted as described in section 3.6 and growth parameters calculated as indicated in section 4.3. In addition cross tabulations and Chi-square tests were used to examine significant differences ($P< 0.05$) in the sex ratios of *O. andersonii* among the treatments (MT levels) and between external sexing using genital papillae and gonadal technique.

7.4 Results

7.4.1 Growth of sex reversed *O. andersonii*

Significant differences ($P< 0.05$) were observed in the FMW (g), BWG (g), SGR (%day^{-1}) and AFCE (%) after 30 days of weaning the fish from MT incorporated feed. The fish subjected to 40 mgMT/kg had the highest FMW (3.121 ± 0.092g) although the highest weight gain was observed in the control group. Similarly, the SGR (%day^{-1}) (7.834 ± 0.155% day^{-1}) was significantly highest ($P< 0.05$) in the control group followed by the 60 mgMT/kg fed group. The rest of the calculated parameters were not significant ($P > 0.05$) after 60 days. The highest SGR (%day^{-1}) was found in the control group although this was not significant different ($P> 0.05$) from the fish fed with 40 mgMT/kg and 90 mgMT/kg (Table 7.3). Ninety days after weaning the fish from MT incorporated feed, all growth indices with an exception of SGR (%day^{-1}) where not significant ($P> 0.05$). The SGR (%day^{-1}) for the control group was significantly higher ($P< 0.05$) than 40 mgMT/kg and 90 mgMT/kg but not significant different ($P > 0.05$) from the 60 mgMT/kg fed *O. andersonii*. The AFCE (%) was similar ($P> 0.05$) among all the treatments (Table 7.3).

Table 7.3: Growth and feed indices of fish fed different levels of MT (mean ± SE)

Parameter	0 mgMT/kg	40 mgMT/kg	60 mgMT/kg	90 mgMT/kg
After 30 days				
FMW (g)	2.656±0.099^{a}	3.121±0.092^{b}	2.863±0.082^{b}	2.601±0.075^{a}
BWG (g)	2.695±0.084^{c}	2.053±0.094^{a}	2.634±0.010^{c}	2.635±0.07^{b}
SGR (%day^{-1})	7.834±0.155^{c}	5.737±0.153^{a}	7.045±0.172^{b}	6.605±0.153^{b}
AFCE (%)	1727.145±102.409^{c}	839.784±47.441^{a}	1349.180±76.841^{b}	1143.050±64.471^{b}
After 60 days				
FMW (g)	5.311±0.184	5.211±0.144	4.993±0.138	4.840±0.169
BWG (g)	4.588±0.233	4.844±0.145	4.585±0.138	4.477±0.170
SGR (%day^{-1})	8.292±0.216^{b}	8.201±0.153^{b}	7.628±0.124^{a}	7.852±0.139^{ab}
AFCE (%)	118.122±6.112	106.138 ± 3.687	107.789±3.628	116.875±5.380
After 90 days				
FMW (g)	6.062±0.262	6.868±0.204	6.713±0.225	6.244±0.279
BWG (g)	5.759±0.264	6.444±0.205	6.331±0.224	5.856±0.276
SGR (%day^{-1})	3.310±0.071^{b}	3.075±0.041^{a}	3.198±0.054^{ab}	3.045±0.054^{a}
AFCE (%)	63.721±3.217	60.724±1.940	62.788±2.379	67.221±3.406

Different superscripts in a row indicate significant difference ($P < 0.05$).

7.4.2 Sex reversal evaluation

None of the treatment produced 100% males. The result of the study revealed no significant differences ($\chi^2 = 2.561$; df = 1; $P > 0.05$) between hand sex (males = 52.2% and females = 42.9%) (Figure 7.4) and microscopic gonadal examination (males = 47.1% and females = 52.9%) (Figure 7.5a) removing intersex gonads (Figure 7.5b), lost gonads and those that could not be identified visually.

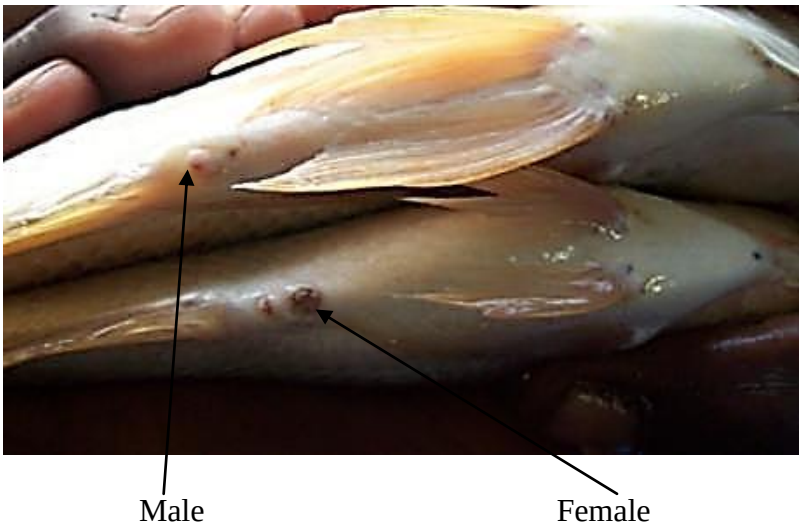


Figure 7.4: Genital papilla for *O. andersonii*

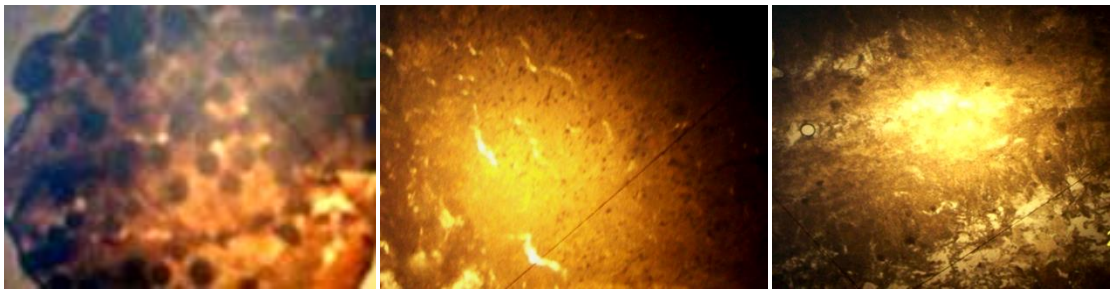


Figure 7.5: Female ovary (a), testicular tissue (b) in gonadal squash and intersex (c) gonad in gonadal squash

The results of the present study showed that each hormone treated group was significantly different ($P< 0.05$) from the control group. Chi square test showed that the number of males from the group treated with 40 mgMT/kg did not differ significantly ($P> 0.05$) from the 60 mgMT/kg treated fish. However, the number of *O. andersonii* males treated with 90 mgMT/kg was significantly lower ($P< 0.05$) than the 40 and 60 mgMT/kg groups but significantly higher ($P< 0.05$) than the control group (Table 7.4).

Table 7.4: Percentage of males, females and intersex according to the MT dose

Treatment	Male (%)	Female (%)	Intersex (%)
Control	54.3^{a2}	44.7^{c2}	1.0^{a1}
40 mgMT/kg	93.4^{c2}	2.2^{a1}	4.4^{ab1}
60 mgMT/kg	94.4^{c2}	3.3^{a1}	2.2^{a1}
90 mgMT/kg	79.3^{b3}	14.6^{b2}	6.1^{b1}

Different letters (superscripts) in a column indicate significant difference ($P < 0.05$) within the dose (treatment). Different numbers (superscripts) in a row indicate significant difference ($P < 0.05$) in the gender of the fish within the treatment.

7.5 Discussion

The experiment shows that fish raised on 40 mgMT/kg and 60 mgMT/kg feed for the first 30 days after weaning had the highest final body weight. The control group and that of fish given 90 mgMT/kg feed had similar weights ($P> 0.05$) among the treatments. Subsequent sampling revealed that FMW (g), BWG (g) and AFCE (%) were not significant ($P> 0.05$) but SGR (%day^{-1}) seemed to favour the control group followed by the 90 mgMT/kg fed fish. The experiment in chapter 6 showed that 60 mgMT/kg feed produced fish with the highest FMW. However, in that experiment fish were not weaned from MT. In an experiment conducted by Pechsiri and Yakupitiyage (2005) found that there were no significant differences ($P> 0.05$) in FMW (g), SGR (%day^{-1}), FCR and survival rate of sex reversed diploid and triploid *O. niloticus*. Similar results were found on Muskellunge, *Esox masquinongy* (Rinchard *et al.*, 1999).

However, the results are inconsistence with those by Mateen (2007). In his study he found significant differences in the growth rate with *O. niloticus* subjected to 70 mgMT/kg feed for 25 days and grown for another 280 days having the highest growth rate. However, there was no attempt to verify if the differences were as a result of anabolic or masculinisation effects. The differences may be due to heterogeneity in the environmental and experimental conditions. Marjani *et al*. (2009) found significant differences in the growth of *O. mossambicus* 42 days after weaning from the feed incorporated with MT. The results therefore show that the anabolic effect imposed by the androgen MT declines as the fish grows.

Several factors (fish age and size, treatment duration, environment and dose rate) have been mentioned to affect sex reversal (Phelps and Popma, 2000). In the current experiment none of the treatment achieved 100% male proportion. This is consistent to other authors (Pelps *et al*., 1992; Green and Teichert – Coddington, 1994; Das *et al*., 2011) who set their experiments in hapas similar to the current study. However, Tayamen and Shelton (1978) and Varadaraj and Pandian (1989) achieved 100% males in *O. niloticus* and *O. mossambicus* using the steel tank and aquarium, respectively. Therefore, treatment environment might be one of the reasons why 100% male proportion was not achieved in the in fish subjected to MT in hapas.

The results on sex reversal experiment showed that hand sexing was insignificant ($P > 0.05$) from gonadal squash examination although the number of males were overestimated by the former method. Using 11 – KT, Carlisle *et al*. (2000) found the

female *Lythrypnus dalli* to have developed a male – like genital papilla within five days of androgen implantation. They further observed no significant ($P > 0.05$) change in the area around the genital papilla suggesting rearrangement of the tissue rather than a reduction or addition in the tissue. This could be due to the mediation of the genital papilla due to gonadal steroids (Oliveira and Almada, 1998). Therefore, the insignificant difference ($P > 0.05$) between the hand sexing and gonadal squash methods may be due to the rearrangement of the genital papilla. Further, the insignificance difference ($P > 0.05$) provides evidence of identifying the sex reversed fish without using laboratory procedures such as gonadal squash method.

The results of the experiment reveal that the proportion of males in MT treated groups deviated significantly from the control group. However, the percentage of males obtained from the 40 (93.4%) and 60 mgMT/kg (94.4%) fish was insignificant ($P > 0.05$). The proportion of males is consistent to what was reported by Pelps *et al*. (1992) (97.8% males), and Green and Teichert – Coddington (1994) (96.8% males) on *O. niloticus* who set their experiment in hapas for 28 days at 60 mgMT/kg feed the protocol similar to the current study. Shepperd (1984) and Celik *et al.* (2011) found similar results on Red tilapia in steel tank and aquaria on *O. niloticus*, respectively. Romerio *et al*. (2000) too found similar results despite feeding *O. niloticus* with feed incorporated with MT for 45 days. The study is also consistent to the findings of Das *et al*. (2011) who observed the highest male percentage (96%) in *O. niloticus* at 60 mgMT/kg feed.

Furthermore, the results showed a significantly lower ($P< 0.05$) male proportion for the highest dose (90 mgMT/kg) used in the experiment. This is consistent to what was reported by Okoko (1996) on *O. niloticus* who obtained 99.3% males at 30 mgMT/kg feed but only 52% at 1, 200 mgMT/kg feed. Marjani *et al*. (2009) found similar results on *O. mossambicus*. In their experiment 98% males were produced when the fish were fed 75 mgMT/kg but only 79.4% males at 100 mgMT/kg. Das *et al*. (2011) had 96% males on 60 mgMT/kg dose but 78.33% at 120 mgMT/kg feed. In the current experiment highest MT dose produced only 79.3% males compared to 94.4% males at 60 mgMT/kg feed. This could be as a result of biosynthesis of excessive androgens into estrogens by the steroidogenic enzyme cytochrome P450 aromatase through the process of aromatisation (Afonso *et al*., 2001).

The results of this experiment show that the anabolic effect of androgen MT diminishes over time and that the effect of MT on fish during masculinisation may not be the factor for faster growth in the sex reversed fish. Furthermore, the study reveals that the 60 mgMT/kg feed produces the highest proportion of males although this is not significant different ($P> 0.05$) from the 40 mgMT/kg fed fish.

CHAPTER 8

GENERAL CONCLUSIONS AND RECOMMENDATIONS

8.1 General conclusions

The 20CL40CP treatment was not different ($P > 0.05$) from all other combinations with 30 and 40% CP. Gross Margin and TC were highest too at the highest lipid and protein combinations. The study provides evidence that 40%CP resulted into the highest final fish weight and economical returns but this was not significant different from the 30%CP. There were no significant differences ($P > 0.05$) in the fish growth rate at any lipid level used although polynomial regression showed 15.3% to give the highest body weight gain.

The study shows that fecundity and GSI could be maximized at 15CL20CP while the egg size at the 15CL40CP combination. However, incubation or brooding number of eggs was maximized at the highest protein (40%) and lipid (20%) levels used in the experiment. The experiment also shows that *O. andersonii* females can spawn without the presence of males. Furthermore, the study shows that females mature earlier than males. Fast growing fish seem to mature earlier than slow growing fish.

The study further revealed that growth of *O. andersonii* was maximized when the feed was incorporated with the 60 mgMT/kg although the highest GM was observed in the control group (without MT). This shows that although there is a biological increase in growth of the fish as a result of hormone incorporation, the increase is marginal to affect any economic advantage.

The study shows that the oral administration of MT does not affect the physiology of *O. andersonii* as the haematology and histopathology of the liver and heart were similar to that of the control. The safety of MT at levels below 60 mgMT/kg to *O. andersonii* is, therefore, proved under the condition of the current experiment similar to the adopted sex reversal practices in other fish species. However, they are signs of condition deterioration as observed in the anaemic conditions in fish fed at 90 mgMT/kg feed treatment.

The study further reveals that the anabolic effect of MT diminishes on *O. andersonii* with time. The superior growth of sex reversed *O. andersonii* cannot, therefore, be attributed to the growth enhancement of MT but probably by masculinisation effect.

The 60 mgMT/kg feed produces the highest percentage of the males although this dose is not significant different ($P > 0.05$) from the 40 mgMT/kg feed.

8.2 General recommendations

The following are proposed:

I. In growing *O. andersonii*, the combinations of 10% lipid and 30% crude protein should be used as it is gives the best growth economically. However, there is need for a follow up study to test the feed on fish over a long period of time at least four months which usually represent the growing period. The methods of feeding should be studied in order to increase the feed utilization consequently

growth at a reduced operational cost. The type of pellets based on density should be tested on *O. andersonii*.

II. Twenty per cent (20%) crude protein and 15% crude lipid should be used if the objective is to maximize fecundity. However, there must be a study to establish the survival of the eggs and hatchings of the brood stock maintained on the diet. The combination 15CL40CP should be used to maximize the size of the eggs. Similarly, there must be a follow up investigation on the relationship between egg size and fry survival.

III. There is no justification of using the female *O. andersonii* in mono sex culture as they are capable of spawning and brooding and that their ability to mature earlier than males would affect growth.

IV. Although there is evidence of anabolic effect of MT at 60 mg/kg of feed in *O. andersonii* there is no economic benefit of hormonal incorporation in the feed.

V. The 60 mgMT/kg feed is recommended as the dose in the sex reversal programmes of *O. andersonii*. However, a follow –up study is recommended to ascertain whether the yield of the 60 mgMT/kg feed administered to *O. andersonii* would be significantly different ($P< 0.05$) from the 40 mgMT/kg fed *O. andersonii* since the sex ratios for both doses were similar ($P> 0.05$). The efficacy of sex reversal using the androgen MT in *O. andersonii* can easily be determined by hand sexing at fingerling stage. Furthermore, there must be a study to determine the sex ratios of *O. andersonii* at a higher MT doses than the 60 mgMT/kg recommended but not higher than 90 mgMT/kg.

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APPENDICES

Appendix 10.1: Analytical procedure for nitrate determination

I. Measure 5ml of deionised water using a measuring cylinder and transfer to a sample cell (blank test)

II. Measure another 5ml of sample water using a measuring cylinder and transfer to another sample cell

III. Pipette 1ml of nitrite reagent and transfer to each sample cell and shake well (wait for ten minutes).

IV. Place the blank test in the spectrophotometer cell slot and record the reading.

V. Repeat IV for the sample test and record the reading.

VI. The difference from the readings is the value of nitrite in water.

Appendix 10.2: Mann – Whitney U test results between the pairs of treatments

Appendix 10.2a: Mann – Whitney U test between the pairs of treatments

Treatments	**Mean Ranks**	**Mann – Whitney U**	***P* – value**
Unfed	12.38	70.000	0.09
10CL20CP	19.32		
Unfed	14.12	92.500	0.097
15CL20CP	18.80		
Unfed	17.77	140.000	0.422
20CL20CP	20.40		
Unfed	13.92	90.000	0.318
10CL30CP	16.71		
Unfed	12.12[b]	66.500	0.002
15CL30CP	20.18[a]		
Unfed	14.12	92.500	0.097
20CL30CP	18.88		
Unfed	14.62	99.000	0.177
10CL40CP	18.55		
Unfed	13.38	83.000	0.059
15CL40CP	18.63		
Unfed	13.12[b]	79.500	0.019
10CL40CP	19.53[a]		

Appendix 10.2b: Mann – Whitney U test results between the pairs of treatments

Treatments	**Mean Ranks**	**Mann – Whitney U**	***P* – value**
10CL20CP	21.45	162.500	0.680
15CL20CP	18.63		
10CL20CP	26.18[a]	167.500	0.032
20CL20CP	19.70[b]		
10CL20CP	20.61	121.500	0.078
10CL30CP	16.15		
10CL20CP	19.45	179.500	0.525
15CL30CP	20.53		
10CL20CP	21.45	162.500	0.245
20CL30CP	18.63		
10CL20CP	21.95	153.000	0.137
10CL40CP	18.15		
10CL20CP	20.50	161.500	0.380
15CL40CP	18.50		
10CL20CP	20.45	181.500	0.680
20CL40CP	19.58		

Appendix 10.2c: Mann – Whitney U test results between the pairs of treatments

Treatments	Mean Ranks	Mann – Whitney U	*P* – value
15CL20CP	24.88	212.5	0.435
20CL20CP	21.50		
15CL20CP	19.88	152.5	0.501
10CL30CP	17.97		
15CL20CP	18.50	160.0	0.080
15CL30CP	22.50		
15CL20CP	20.50	200.0	1.000
20CL30CP	20.50		
15CL20CP	21.0	190.0	0.727
10CL40CP	20.0		
15CL20CP	19.63	182.5	0.773
15CL40CP	20.39		
15CL20CP	19.50	180.0	0.435
20CL40CP	21.50		

Appendix 10.2d: Mann – Whitney U test results between the pairs of treatments

Treatments	Mean Ranks	Mann – Whitney U	*P* – value
20CL20CP	21.10	202.50	0.761
10CL30CP	22.09		
20CL20CP	19.50[b]	162.50	0.007
15CL30CP	27.38[a]		
20CL20CP	21.50	212.50	0.294
20CL30CP	24.88		
20CL20CP	22.00	225.00	0.491
10CL40CP	24.25		
20CL20CP	20.70	192.50	0.186
15CL40CP	24.87		
20CL20CP	20.50	187.5	0.069
20CL40CP	26.13		

Appendix 10.2e: Whitney U test results between the pairs of treatments

Treatments	Mean Ranks	Mann – Whitney U	*P* – value
10CL30CP	15.97[b]	118.500	0.021
15CL30CP	21.58[a]		
10CL30CP	17.97	152.500	0.501
20CL30CP	19.88		
10CL30CP	18.47	161.000	0.735
10CL40CP	19.45		
10CL30CP	17.15	138.500	0.348
15CL40CP	19.71		
10CL30CP	16.97	135.500	0.157
20CL40CP	20.73		

Appendix 10.2f: Mann – Whitney U test results between the pairs of treatments

Treatments	Mean Ranks	Mann – Whitney U	*P* – value
15CL30CP	22.500	160.000	0.080
20CL30CP	18.500		
15CL30CP	23.000[a]	150	0.040
10CL40CP	18.000[b]		
15CL30CP	21.530	159.500	0.139
15CL40CP	18.39		
15CL30CP	21.50	180	0.298
20CL40CP	19.50		

Appendix 10.2g: Mann – Whitney U test results between the pairs of treatments

Treatments	Mean Ranks	Mann – Whitney U	*P* – value
20CL30CP	21.000	190.00	0.727
10CL40CP	20.000		
20CL30CP	19.630	182.500	0.773
15CL40CP	20.390		
20CL30CP	19.500	180.00	0.435
20CL40CP	21.500		

Appendix 10.2h: Mann – Whitney U test results between the pairs of treatments

Treatments	Mean Ranks	Mann – Whitney U	*P* – value
10CL40CP	19.150	173.000	0.528
15CL40CP	20.890		
10CL40CP	19.000	170.000	0.262
20CL40CP	22.000		

Appendix 10.2i: Mann – Whitney U test results between the pairs of treatments

Treatments	Mean Ranks	Mann – Whitney U	*P* – value
15CL40CP	19.390	178.500	0.627
20CL40CP	20.580		

Appendix 10.3: Number of immature and mature *O. andersonii* according to treatments

Maturity stage of the fish		Treatment										
		Unfed	10CL20CP	15CL20CP	20CL20CP	10CL30CP	15CL30CP	20CL30CP	10CL40CP	15CL40CP	20CL40CP	Total
Immature	Count	7	2	5	10	6	1	5	6	4	3	49
	Expected count	3.3	4.8	5.1	6.3	4.3	5.1	5.1	5.1	4.8	5.1	49.0
	% within maturity stage of the fish	14.3	4.1	10.2	20.4	12.2	2.0	10.2	12.2	8.2	6.1	100.0
Mature	Count	6	17	15	15	11	19	15	14	15	17	144
	Expected count	9.7	14.2	14.9	18.7	12.7	14.9	14.9	14.9	14.2	14.9	144.0
	% within maturity stage of the fish	4.2	11.8	10.4	10.4	7.6	13.2	10.4	9.7	10.4	11.8	100.0
Total	Count	13	19	20	25	17	20	20	20	19	20	193

χ^2 = 17.416, df = 9, *P*< 0.05

Appendix 10.4: Correlations among the nutrients, feed utilization and growth parameters

		Protein Nutrient level	**Total cost (ZK)**	**Gross margin**	**Lipid nutrient level**	**Body gain weight**	**Specific growth rate**	**Apparent feed conversion efficiency**
Protein Nutrient level	R	1	0.582	0.438	0.561	0.462	0.352	0.249
	P– value	-	0.000	0.000	0.000	0.000	.000	0.000
	N	298	298	273	298	298	273	298
Total cost (ZK)	R	0.582	1	0.122	0.539	0.060	-0.355	-0.407
	P – value	0.000	-	0.043	.000	.300	.000	0.000
	N	298	298	273	298	298	273	298
Gross margin	R	0.438	0.122	1	0.257	0.983	0.701	0.673
	P – value	0.000	0.043	-	0.000	0.000	0.000	0.000
	N	273	273	273	273	273	273	273
Lipid nutrient level	R	0.561	0.539	0.257	1	0.291	0.216	0.110
	P– value	.000	.000	0.000	-	0.000	0.000	0.058
	N	298	298	273	298	298	273	298
Body gain weight	R	0.462	0.060	0.983	0.291	1	0.798	0.736
	P – value	0.000	0.300	0.000	0.000	-	0.000	0.000
	N	298	298	273	298	298	273	298
Specific growth rate	R	0.352	-0.355	0.701	0.216	0.798	1	0.937
	P – value	0.000	0.000	0.000	.000	0.000	-	0.000
	N	273	273	273	273	273	273	273
Apparent feed conversion efficiency	R	0.249	-0.407	0.673	.110	0.736	0.937	1
	P– value	0.000	0.000	.000	.058	0.000	0.000	-
	N	298	298	273	298	298	273	298

Appendix 10.5: Largest fish isolated from each protein and lipid combination

Appendix 10.6: Publications and presentations from the project

- **Kefi, A. S., Kang'ombe, J., Kassam, D and Katongo, C. 2013.** Effect of Dietary Soyabean (*Glycine max* (L.) Merr.) Protein Level on Growth and Feed Utilization of *Oreochromis andersonii* (Castelnau, 1861). *Pakistan Journal of Nutrition* 12 (11): 990 – 995.

- **Kefi, A. S., Kang'ombe, J., Kassam, D and Katongo, C. 2013**. Effect of 17 α – Methyl Testosterone on Haematology and Histology of Liver and Heart of *Oreochromis andersonii* (Castelnau, 1861). *Journal of Marine Science Research and Development* 3: 1- 7 http://dx.doi.org/10.4172/2155-9910.1000130.

- **Kefi, A. S., Kang'ombe, J., Kassam, D and Katongo, C. 2013.** Optimal Dietary Plant Based Lipid on Growth of *Oreochromis andersonii* (Castelnau, 1861). Turkish Journal of Fisheries and Aquatic Sciences 13: 503 – 508. DOI: 10.4194/1303-2712-v13_3_13.

- **Kefi, A. S., Kang'ombe, J., Kassam, D and Katongo, C. 2012.** Growth, reproduction and sex ratios in *Oreochromis andersonii* (Castelnau, 1861) fed with varying levels of 17α – methyl testosterone. Journal of Aquaculture Research and Development 3: 1 - 7 http://dx.doi.org/10.4172/2155-9546.1000155

- **Kefi, A. S., Kang'ombe, J., Kassam, D and Katongo, C. 2011.** The Effect of 17α – Methyltestosterone (MT) on the Growth and Reproductive Characteristics of *Oreochromis andersonii* (Castelnau, 1861). Aqua Africa Conference, Septermber 13 – 16, 2011, Mangochi, Malawi.

- **Kefi, A. S., Kang'ombe, J., Kassam, D and Katongo, C. 2011.** Soya Bean Protein and Lipid Combinations on Growth and Feed Efficiency of *Oreochromis andersonii*. 10th African Crop Science Society Conference. October 10 – 13, 2011, Maputo, Mozambique.