EFFECT OF DIETARY PROTEIN LEVEL ON THE GROWTH RATE OF NILE TILAPIA (Oreochromis niloticus L.) FINGERLINGS.

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DEDICATION

I dedicate this report to my family for their support throughout my academic life.

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My special regards go to Kenya Marine and Fisheries Research Institute-Sangoro station for allowing me to carry out my experiment at their station and facilitating it.

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ABSTRACT

The influence of commercial feeds on growth performance of Nile Tilapia (Oreochromis niloticus) fingerlings was investigated in trial lasting 21 days. 100 fingerlings were maintained in hapa nets in triplicate. Fish feed containing 15%, 25% and 35% crude protein(CP) was formulated with ingredients including fresh water shrimp, sunflower seed cake and wheat pollard. Fish were fed equivalent 3 % of their wet body weight twice daily at 12 hours interval for 4 weeks.

Fish growth parameters (length and weight) were taken after every two weeks. Water quality parameters which included pH, temperature, dissolved oxygen and salinity were checked daily to ensure that they were optimum. Mortality rate was also checked daily in the three hapas.The water quality parameters were also recorded weekly.

Results revealed that increasing dietary crude protein increased final body weight (FBW) significantly ($P < 0.001$). Fish that were maintained at 35% protein levels exhibited significantly higher FBW compared to those fed 15 and 25%. Based on weight gain and body length it could be suggested that 35% dietary crude protein is optimum for Nile Tilapia in hapa nets, using commercial ingredients.

CHAPTER ONE

1. INTRODUCTION

Tilapia spp are considered as the best species for culture due to their high tolerance to adverse environmental conditions, their relatively fast growth rate and their ease of breeding (El-Sayed, 1999). Tilapia intensive culture requires the formulation of efficient feed with optimum potency to meet the protein requirements during early growth period (Kenawy, 1993).

Tilapia rearing has several benefits to the Kenyan economy:

- **Food security**

In Kenya, the per capita annual consumption of fish is 2.8 kilograms compared to the worldwide average of 16 kg. (FAO, 2005.)Fish contributes 11% of average daily protein consumption throughout the country.(ADB;2005)Kenya has tremendous access to both freshwater, Lake Victoria, and marine fisheries;

- **Economic Role of Fisheries**

In 2005, fisheries contributed to 27.4% of the agricultural GDP for Kenya. Lake Victoria accounts for 92% of fisheries production in Kenya while marine capture fisheries consist of 4% of the total national catch for Kenya. In 2005, the gross value of Kenyan fisheries landing was US$104,500,000 and fisheries exports were worth US$ 49,684,000.Other benefits for fish rearing include job creation and poverty reduction strategy.

During Tilapia production, feed cost is considered as a major part of the variable costs, and protein is the most expensive component of the feed. Thus reduction of protein in tilapia feed is of interest to fish nutritionists. If a feed has insufficient energy or has excess amount of protein in relation to the concentration of dietary energy, the extra protein will be used as a source of energy (Phillips, 1972; Prather and Lovell R.T; 1973). Several studies have shown that when lipid is a source of energy there is a sparing action on protein and could be used in the fish feed as an energy source. This will minimize the use of protein as a source of energy (Ringrose, 1971; Lee and Putnam, 1973; Watanabe. 1977); however, increasing energy may produce fatty fish, reduce feed consumption and inhibit the utilization of other feed stuffs (Page and Andrews, 1973).

Formulation of diets for any fish species requires understanding of the nutritional requirements of the species. However, minimum crude protein in tilapia feed depends on the rearing system used (Twibell and Brown; 1998).

Protein requirements for optimum growth of the fish seem to be affected by numerous factors such as temperature, salinity, fish age and size, among others. (Cowey, 1976). Most nutritional studies have been confined to diseased and mature fish although the supplementary feed has been used during fingerlings and fry phase. Furthermore, understanding the protein requirement during the early growing period is an important aspect in fish culture management. Realization of the optimum protein level for cultured fish would help reduce the costs and maximize the feed conversion efficiency (Charles et al., 1984; Sampath, 1984).This study was initiated to evaluate effects of varying dietary crude protein levels from 15-35% on growth rate of Nile Tilapia fingerlings.

2. JUSTIFICATION

Commercial fish feeds are very expensive and difficulty to access hence the need for low cost feed without affecting growth. Nile Tilapia is a very popular fish that fetches high market price. Its commercial production in ponds is a very profitable enterprise. Details of the nutritional requirements for the different developmental stages of Nile Tilapia and adaptations to different diets that would enable development of these diets are not clear.

3. OBJECTIVES

General objective

Achieve low cost feed from readily available materials with good effect on growth.

Specific objective

Assess the growth rate of Tilapia fingerlings fed on different levels of protein.

CHAPTER TWO

4. LITERATURE REVIEW

4.1. History of origin of Nile Tilapia

The Nile tilapia, *Oreochromis niloticus,* is well known for its wide use in augmenting natural fisheries and for fish farming. It is not a native of the Lake Victoria basin of East Africa. Nevertheless, it has become the most dominant tilapia species in the Lake Victoria region and is Second only to another introduced species, the Nile perch, *Lates niloticus*, in economic importance in the region (Ogutu-Ohwayo 1990; Balirwa 1992; Stiassny 1996). In the Lake Victoria region, exploitation of the species is still largely from populations in natural waters. The Nile tilapia invaded Lake Victoria in the early 1900s, with the first recordings of the species in the lake occurring in the 1920s (Trewavas 1983). Trewavas postulates that *O. niloticus* may have entered the lake through the Kagera River, following introductions into Lake Bunyonyi from Lake Edward. According to Fryer and Iles (1972), intentional introduction of *O. niloticus* into the Lake Victoria basin may first have occurred in the late 1930's, following the repeated failure of attempts to introduce *Tilapia* (*Oreochromis*) into the Koki lakes, a part of the Lake Victoria basin southwest of Lake Victoria. *Tilapia* (*Oreochromis*) *niloticus* was introduced in the Koki lakes, immediately became successfully established, and continues to flourish and dominate the Koki lakes. This success provided a lesson in the versatility of *O. niloticus* for fisheries managers. Subsequently, *O. niloticus* was introduced into virtually all significant water bodies in Kenya (Fryer 1972; Fryer and Iles 1972).

4.2. History of domestication

Nile tilapia has been farmed for centuries. Depictions on an Egyptian tomb (dated at 4000 years) display the fish in ornamental ponds. The culture of the tilapia genus on a global scale, primarily *Oreochromis mossambicus*, began in the 1940s. However, it was not until the 1960s that *O. niloticus* was exported worldwide (FAO 2012).

Aquaculture was heralded as the perfect protein production technique for developing countries during the 1960s and 1970s. Aid organizations promoted aquaculture as a means of improving food security with low grain to feed conversion rates, and minimal environmental impacts (Canonico *et al.* 2005).

This global popularity has led to a number of important developments in culture techniques. Initially, tilapias were allowed to breed freely. However, farmers and scientists observed that this led to the production of smaller fish. In the 1960s, attempts were made to produce male monosex populations through hybridization between different tilapia species (Hickling 1963). This proved problematic and gradually females reappeared in the progeny (Wohlfarth 1994). Major technological developments in the 1970s allowed for the successful production of all-male populations through the use of sex-reversing hormones which resulted in higher returns from tilapia farming. Following this, and further research into culture processes, the industry boomed (FAO 2012).

Natural distribution and habitat

O. niloticus is native to central and North Africa and the Middle East (Boyd 2004) (Figure 2). It is a tropical freshwater and estuarine species. It prefers shallow, still waters on the edge of lakes and wide rivers with sufficient vegetation (Picker & Griffiths 2011).

4.3 Biology of Nile Tilapia

The Nile tilapia *Oreochromis niloticus* is a deep-bodied fish with cycloid scales. Silver in color with olive/grey/black body bars, the Nile tilapia often flushes red during the breeding season (Picker & Griffiths 2011) . It grows to a maximum length of 62 cm, weighing 3.65 kg (at an estimated 9 years of age) (FAO 2012). The average size (total length) of *O. niloticus* is 20 cm (Bwanika *et al.* 2004). The Nile tilapia is still the most widely cultured species of tilapia in Africa (SRAC publication, march 1999).

Figure. Image of the *Oreochromis niloticus* (Source: FAO 2012)

Positive aqua cultural characteristics of tilapia are their tolerance to poor water quality and the fact that they eat a wide range of natural food organisms. Biological constraints to the development of commercial tilapia farming are their inability to withstand sustained water temperatures below 50 to 52^0 F and early sexual maturity that results in spawning before fish reach market size (Aquaculture Collaborative Research Support Program (ACRSP) 2007).

4.3.1.Life cycle

Fish life cycles vary among species. In general, however, fish progress through the following life cycle stages:

• **Eggs:** Fertilized eggs develop into fish. Most eggs do not survive to maturity even under the best conditions. Threats to eggs include changes in water temperature and oxygen levels, flooding or sedimentation, predators and disease.

• **Larval fish:** Larval fish live off a yolk sac attached to their bodies. When the yolk sac is fully absorbed the young fish are called fry.

• **Fry:** Fry are ready to start eating on their own. Fry undergo several more developmental stages, which vary by species, as they mature into adults. Young fish are generally considered fry during their first few months (during their first few months to less than one year in some species).

• **Juvenile:** The time fish spend developing from fry into reproductively mature adults varies among species. Most fish do not survive to become adults. Threats to survival include fluctuations in water temperature, changes in oxygen levels, competition for habitat, and predators. This are also referred to as fingerlings and they are the focus of this study.

• **Adult:** When fish are able to reproduce, they are considered adults. The time it takes to reach maturity varies among species and individual fish.

4.3.2. Taxonomy

Tilapia is the generic name of a group of cichlids endemic to Africa. The group consists of three aquaculturally important Oreochromis, Sarotherodon and Tilapia (SRAC publication, 1999).Several characteristics distinguish these three genera, but possibly the most critical relates to reproductive behavior (Kenya Aqua manual, 2007). All tilapia species are nest builders; fertilized eggs are guarded in the nest by a brood parent. Species of both Sarotherodon and Oreochromis are mouth brooders; eggs are fertilized in the nest but parents immediately pick up the eggs in their mouths and hold them through incubation and for several days after hatching. In Oreochromis species only females practice mouth brooding, while in Sarotherodon species either the male or both male and female are mouth brooders (Kenya Aqua manual, 2007).

The scientific names of tilapia species have been revised frequently in the last 30 years, creating some confusion. The scientific name of the Nile tilapia has been given as Tilapia nilotica, Sarotherodon niloticus, and currently as Oreochromis niloticus (Thomas Popma and Michael Masser, 1999).

4.3.3. Reproduction

In all Oreochromis species the male excavates a nest in the pond bottom (generally in water shallower than 3 feet) and mates with several females. After a short mating ritual the female spawns in the nest (about two to four eggs per gram of brood female), the male fertilizes the eggs, and she then holds and incubates the eggs in her mouth (buccal cavity)until they hatch. Fry remain in the females' mouth through yolk sac absorption and often seek refuge in her mouth for several days after they begin to feed (Leo and Schofield, 2008).Sexual maturity in tilapia is a function of age, size and environmental conditions (Hecht and Endemann, 1998).

4.4. Feeding habits and nutrition

Nile tilapia is omnivorous, feeding lower on the food chain on phytoplankton, zooplankton, aquatic insects, and macrophytes. Protein requirements for maximum growth are a function of protein quality and fish size and have been reported as high as 50 percent of the diet for small fingerlings (Linnaeus, 1757).

4.5. Environmental requirements

Salinity

The Nile tilapia is the least saline tolerant of the commercially important species, but grows well at salinities up to 15 ppt. Nile tilapias can reproduce in salinities up to 10 to 15ppt, but perform better at salinities below 5 ppt. Fry numbers decline substantially at 10 ppt salinity

Water temperature

The intolerance of tilapia to low temperatures is a serious constraint for commercial culture in temperate regions. Optimal water temperature for tilapia growth is about 85 to 88oF Growth at this optimal temperature is typically three times greater than at 72o F.

pH

In general, tilapia can survive in pH ranging from 5 to 10 but do best in a pH range of 6 to 9.

Dissolved oxygen concentration

Tilapia survives dissolved oxygen (DO) concentrations of less than 0.3 mg/L, considerably below the tolerance limits for most other cultured fish. In research studies by Trewavas(1983) Nile tilapia grew better when aerators were used to prevent morning DO concentrations from falling below 0.7 to 0.8 mg/L compared with unaerated control ponds (Trewavas, 1983). Growth was not further improved if additional aeration kept DO and other concentrations above 2.0 to 2.5 mg/L.

4.6. Protein supplementation

Studies have been done for grow out period using different initial body weights and varying crude protein levels in feed (Mohammad H.Ahmad1-2000).Studies have also been done on effect of protein and energy levels in commercial diets on growth performance of juvenile Nile tilapia (Oreochromis niloticus) (Alaa El Dahhar-2000).The results showed that feed intake increased as dietary protein increased, however dietary energy seemed to have no influence on this trait.

Protein requirements of tilapia have been extensively studied using dose-response procedures. In this regard, semi-purified test diets containing casein, casein/gelatin mixtures or casein/amino acid mixtures as protein sources or using practical diets in which animal and/or plant ingredients served as dietary protein sources have been widely used. The results of most of these studies are questionable, because they: (1) were conducted indoor, (2) were short-term, (3) may not be directly applied in field trials, and (4) relied mainly on casein (which is deficient in the essential amino acid (EAA) arginine) as a sole dietary protein. Casein/gelatin-based diets were found to be utilized more efficiently than casein/amino acid (AA) diets (El-Sayed, 1989).Thus; it is not surprising that the results of protein requirements of tilapia are varying and sometimes contradictory.

Since purified and semi-purified protein sources are not recommended for tilapia under commercial culture conditions, other conventional and unconventional, locally available dietary protein sources should be sought (Twibell and Brown 1998). Research has evaluated many such sources for different species of tilapia, with varying results (National Research Council 1993).

Therefore, it is appropriate to highlight these protein sources for tilapia, with emphasis on the sources that have economic potential and are locally available, especially in developing countries. A comprehensive review of the possible alternative protein sources for farmed tilapia has been reported by El-Sayed (1999).

4.6.1. Fish meal

Fish meal (FM) has been traditionally used as the main protein source in the aqua feed industry (FAO, 2005). However, the increased demand for FM, coupled with a significant shortage in global FM production has created sharp competition for its use by the animal feed industry (Winfree and Slickney, 1981). As a result, FM has become the most expensive protein commodity in aquaculture feeds in recent years (Tacon, 1993). Many developing countries have realized that, in the long-run, they will be unable to afford FM as a major protein source in aqua feeds.

As such, many attempts have been made to partially or totally replace FM with less expensive, locally available protein sources. The exception is fish silage and shrimp meals, where several studies have considered their use as a FM replacer in tilapia feeds. The results indicated that between 30 to 75% fish silage can be successfully incorporated in tilapia feed, depending on fish species and size, silage source and diet composition (Fagbenro and Jauncey, 1994.). It is evident that fish silage has potential as a protein source for tilapia.

Shrimp meal has also been successfully used as a protein source for tilapia. Blue tilapia(O. aureus), and Nile tilapia utilized shrimp head meal at up to 15% and 60% of the diet without adverse effects on their performance (Toledoet al., 1987; Nwanna and Daramola, 2000).

4.6.2. Cottonseed meal/cake

Cottonseed meal (CSM) is one of the best plant protein sources for tilapia in developing countries, due to its high availability, relatively low price, good protein content (26-54%,depending on processing methods) and amino acid profile (FAO, 1983). However, it is deficient in some EAA such as Cysteine, Lysine and Methionine in addition to its high content of gossypol (a phenolic anti nutrient) that may limit the use of CSM in tilapia feeds. Results on the use of CSM and CSC (cottonseed cake) indicated that replacement of more traditional protein sources at between 50 and 100% can be effective in tilapia feed, depending on CSM source, processing methods and fish species and size(Paraso, (1990).

4.6.3. Economic evaluation of protein sources

Protein sources from oilseed by products such as soybean meal and CSM may result in a significant reduction in fish performance, but may be more cost effective than standard, expensive proteins (such as FM). Therefore, economic evaluation of such protein sources for tilapia is necessary. However, only a few studies have considered both economic and biological evaluation of dietary protein sources for tilapia. These studies demonstrated that sources like CSM (El-Sayed, 1990), corn gluten feed and meal (Wuet al., 1995) animal by-product meal (Rodriguez-Sernaet al., 1996; El-Sayed, 1998) and brewery waste (Oduro-Boateng and Bart-Plange, 1988) can be used as total fishmeal replacers for tilapia despite lower biological performance.

CHAPTER THREE

5. MATERIALS

Nile Tilapia fingerlings were obtained from Kenya Marine and Fisheries Research Institute, at Sangoro station, Kisumu. This is located 1140m above sea level at longitude 034^0N 58.1^0E and latitude 0^042^0N 50.44^0S. Fish were kept in hapa nets in earthen pond for a 3-week experimental period. Nile tilapia fingerlings of initial body weight 1.5 ± 0.13g and length 3.5 ±1.5cm were stocked at a rate of 100 fish per hapa net. The hapa nets with dimensions (84*137x82 cm) were supplemented with good water conditions. Water quality was checked daily, Water temperature was 29^0C. Dissolved Oxygen and water temperature were checked daily using a Cole Palmer {Chicago, Illinois 60648) oxygen meter (model 5946-55). They ranged from 6.5 to 6.9 ppm and from 26.5 to 27.5°C respectively .A weighing scale (bench Carolina scale) was available in the laboratory for taking weight measurements and a cm ruler for length.

Diets formulation and preparation;

Three experimental diets were formulated from commercial ingredients of water shrimps, wheat pollard, and sunflower seedcake to achieve three dietary crude protein levels (15%, 25%and 35%). Dry ingredients were milled through screen (0.6mm diameter) before mixing into the diets. Mixtures were homogenized in a feed mixer (Model SNFGA Kitchen Aid St. Joseph, MI 49085 USA). Boiling water was then added to the mixtures at the rate of 50 % for pelleting. The diets were pelleted and packed in polythene bags until feeding. The Nile Tilapia fingerlings were fed twice a day in the morning and evening.

LIST OF INGREDIENTS AND MIXING RATIO

Feed materials	Amount of feed in diet 1(15%CP)in grams	Amount of feed in diet 2(25%CP)in grams	Amount of feed in diet 3 (35%CP) in grams
Water shrimps(60%CP)	26	9	35
Sunflower seed cake(25%CP)	102	53	38
Wheat pollard(18%CP)	72	38	27

Calculation of feed to be fed each day using average weight.

Hapa 1=average wt 1.28g*3/100*100g=3.84g

Hapa 2=average wt 0.98g*3=2.94g

Hapa 3=average wt 1.26g*3=3.78g

6. METHODOLOGY

The fish was starved for two days prior to their transfer to the hapa nets from the hatchery laboratory. During the transfer I made them adapt to the new pond environment for about 30minutes to avoid shock due to new water conditions and environment. I would feed them twice daily with the research feed I formulated for them. A fish should feed on a meal weighing 3%of its body weight hence I provided feed amount as per that requirement. I would take my time and observe the fish feeding on the pellets to ensure they were of normal behavior and were consuming the diet. Mortality rate at each hapa net was 1% for the first week and on mortality was observed for last two weeks.

Water quality records for the three weeks

	WEEK1	WEEK2	WEEK3
Water Temperature (^{0}C)	21.5	21.8	21.1
Dissolved oxygen (mg/l)	1.70	0.4	0.3
Conductivity(micro Siemen)	524	512	608
Salinity (ppt)	0.1	0.3	0.3

CHAPTER 4

7. DATA ANALYSIS

Data collection was done after every one week .Length and weight measurements were taken from a sample of 20 fish taken randomly from each hapa net. The data recorded was analyzed at the end of the experiment using Genstat computer software.

The growth performance of Nile tilapia fed different protein levels is ' shown in figure 1 and 2.

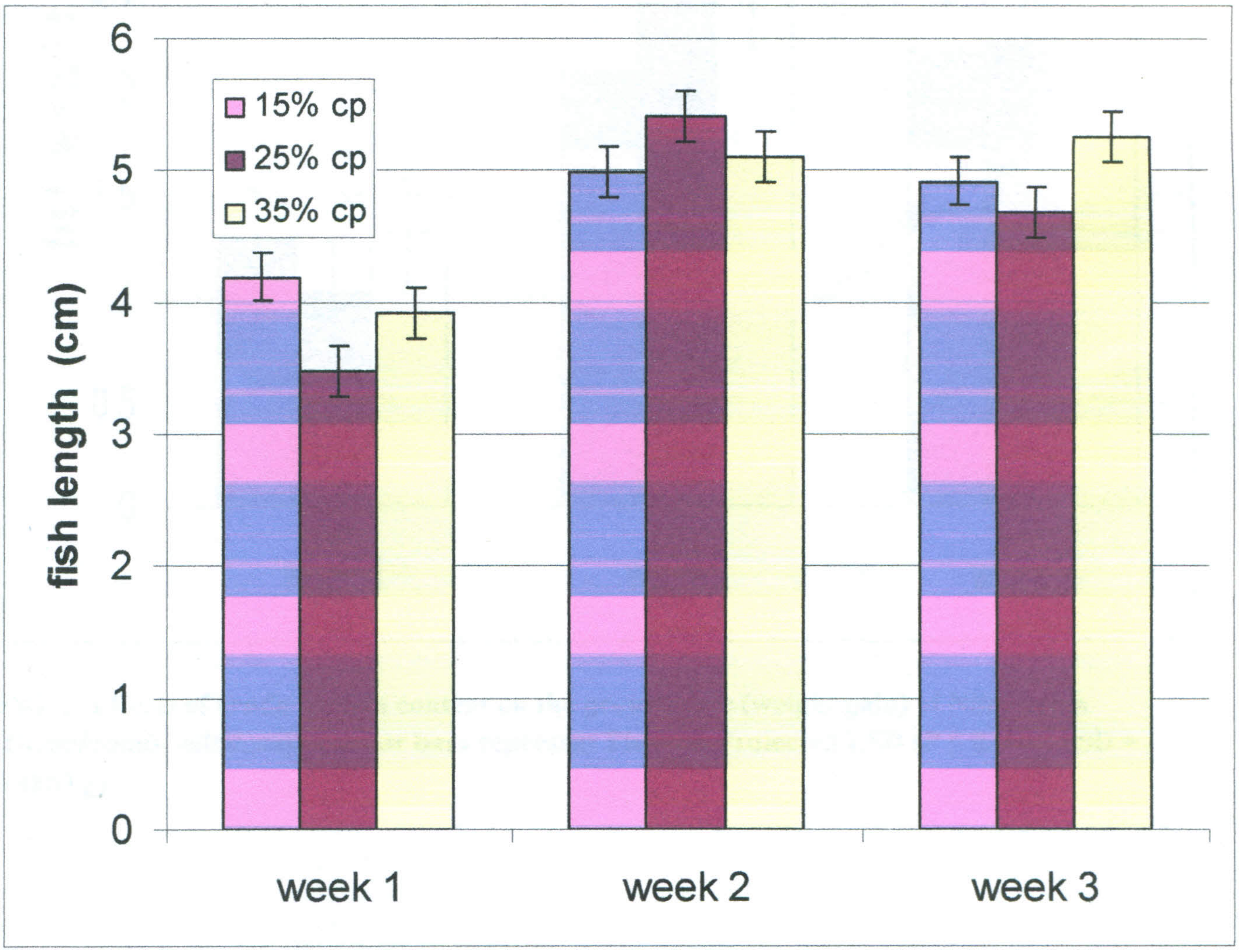

FIG. 1. Effects of crude protein content on the growth rate (increase in length) of Nile tilapia (*Oreochromis niloticus*). Y-error bars represent Fisher's Protected LSD ($P \leq 0.001$; LSD = 0.373 cm).

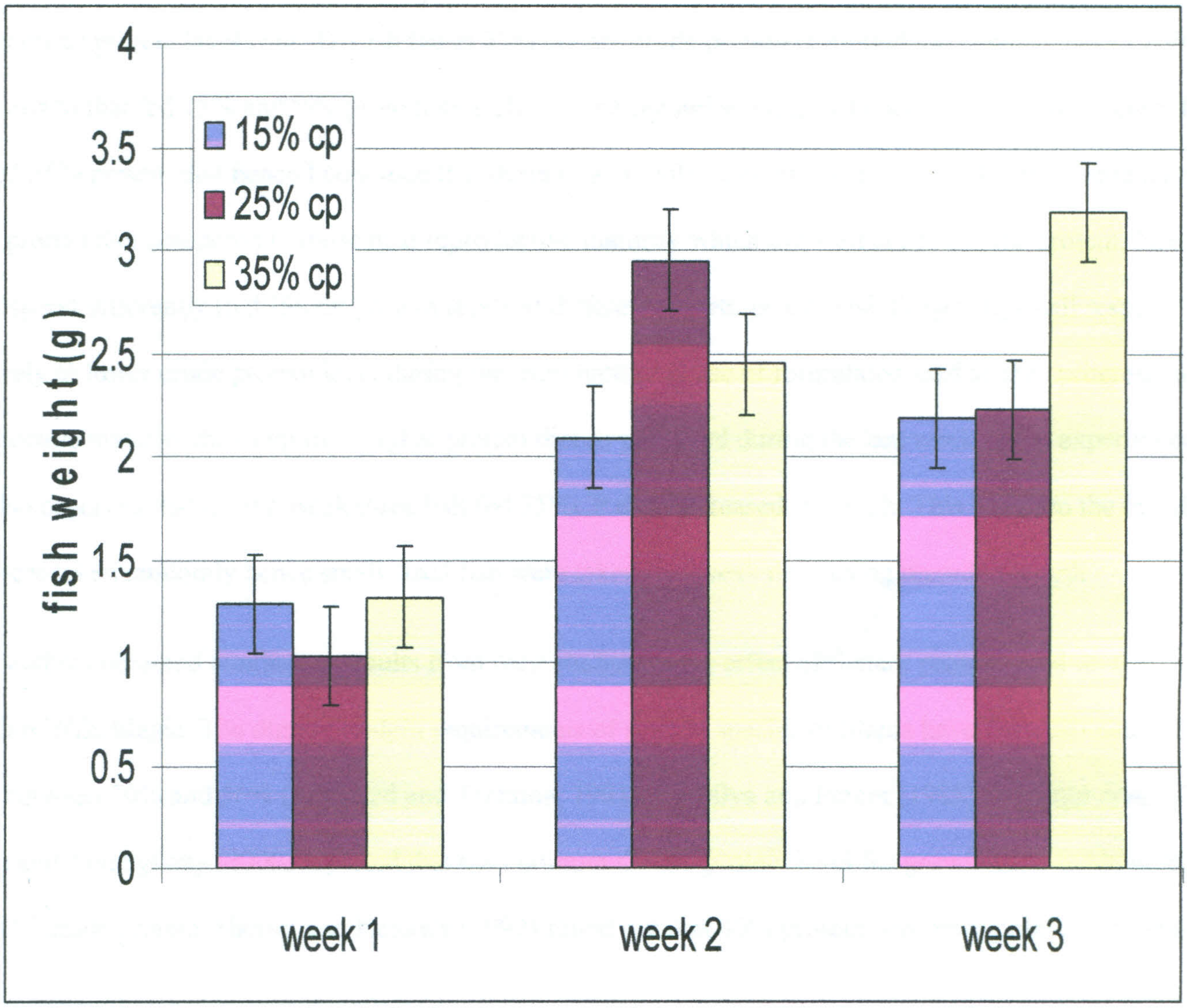

FIG. 2. Effects of crude protein content on the growth rate (weight gain) of Nile tilapia (*Oreochromis niloticus*). Y-error bars represent Fisher's Protected LSD ($P \leq 0.001$; LSD = 0.4853 g)

8. DISCUSSION

Final body weight (FBW) of Nile Tilapia fingerlings increased significantly ($P < 0.001$) with increasing dietary crude protein level (Fig. 2). Fish fed at 35% dietary crude protein exhibited the greatest mean FBW compared to that fed 15% and25% protein diet. During the second week growth performance was highest for fish fed 25% protein diet hence I conclude that during the middle stage of juvenile growth fish require a lower crude protein diet compared to those near reproductive maturity which did well at 35% crude protein. Fish will respond differently to different protein levels at different growth phase. Fish Fingerlings will respond positively to lower crude protein level during the introductory phase of formulated feed as they progress to reproductive maturity they require a higher protein diet as projected during the last week of the experiment. It was also observed that during week three fish fed 25%CP diet decreased in length. This is due to the fact that they were taken randomly hence small sized fish were taken that week decreasing recorded length.

Many authors obtained conflicting results from their studies on the effect of dietary protein level on the growth of Nile tilapia. The dietary protein requirements of several species of tilapia have been estimated to range between 20% and 56% (El-Sayed and Teshima, 1991). De Silva and Perera (1985), Siddiqui *et al.* (1988) and Abdelghany (2000) reported that the optimum dietary protein level for growth of Nile tilapia fry was 30% crude protein. Hamza and Kenawy (1997) found out that 40% protein was more potent than other levels for Nile tilapia growth. Al-Hafedh (1999) and Al-Hafedh *et al.* (1999) found out that the better growth of Nile tilapia was obtained at high dietary protein levels (40-45%) rather than 25-35%. Khattab *et al.* (2000) studied the optimum dietary protein level for Nile tilapia collected from Aswan, Abbassa, Manzalah and Maryut. They found out that the optimum dietary protein level is 37% for Abbassa strain, 27% for Aswan strain and 32% for Manzalah and Maryut strains.

The considerable variations in the results recorded previously for optimum dietary protein requirements for maximum growth might be due to the variations in fish size and age, stocking density, protein quality, hygiene and environmental conditions or other unknown factors, which mask the standardization of the parameters.

CHAPTER 5

9. CONCLUSION

Fish length and weight are significantly influenced by the amount of protein in the diet but the response is influenced by the growth phase. During the first week of feeding formulated diet, fish response is lower to high CP content diet due to stress from adapting to new diet source. Hence feeding low protein and increasing it weekly would give them time to adjust. Environmental conditions and stocking density should be optimum since they affect feed intake and efficiency. These parameters are very important since they determine the general survival of the fish.

10. RECOMMENDATION

I would recommend farmers engaged in fish farming to start with low protein in the first week of feeding commercial feeds and increase it progressively as the fish grows. This would reduce cost incurred due to inappropriate feeding system.

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