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Ibrahim Al-Mohsen

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EFFECT OF GENDER ON NUTRITIONAL REQUIREMENTS OF NILE TILAPIA *OREOCHROMIS NILOTICUS*

By

Ibrahim Al-Mohsen

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ABSTRACT

EFFECT OF GENDER ON NUTRITIONAL REQUIREMENTS OF NILE TILAPIA *OREOCHROMIS NILOTICUS*

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Nile tilapia (*O. niloticus*) males grow faster than females in mixed gender groups under intensive aquaculture because of their early maturation and frequent breeding. This study was designed to determine if the saturation kinetic model could be used to describe weight gain and net nutrient deposition as a function of nutrient intake. Nutrient response curves were developed for female, male, and mixed gender groups fed varying levels of a commercial diet for six weeks. The shape of the curve (n), curve parameters (R_{max}, $K_{0.5}$, b), and calculated maintenance level (ML) varied between gender groups. All-male Nile tilapia gender groups grew faster than female or mixed-gender groups. Males exhibited higher R_{max} and $K_{0.5}$ for growth than female or mixed-gender groups because they used fat, protein and energy more efficiently. Based on our results, methods to provide all-male tilapia for aquaculture should be used to maximize growth and performance.

DEDICATION

This thesis is dedicated with gratitude to my father and mother and my wife Maha without whose encouragement, patience and prayers, this work could never have been completed; I shall be grateful to them to the rest of my life. Dedicated also to brothers, sisters, and other relatives.

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INTRODUCTION

Tilapia[1] have been identified as a major source of animal protein in many developing countries (Pullin and McConnel 1982). These fish were first cultured in Africa around 4,000 years ago by the ancestors of the Egyptians (Lee and Newman 1992). Over the last six decades, tilapia have been introduced in many countries around the world because they are relatively easy to raise under tropical and sub-tropical conditions. The most important species of tilapia for aquaculture have been *Oreochromis aurea*, *O. hornorum*, *O. mossambicus*, *O. niloticus*, *Sarotherodon melantheron*, *Tilapia rendaiii*, and *T. zillii* (Pillay 1990).

Nile tilapia, *O. niloticus*, appears to have gained a wider acceptability than the other species by consumers and fish farmers (Guerrero 1978; Pillay 1990; Landau 1992; Avault 1996; Egna and Boyd 1997). The Nile tilapia continues to be one of the most popular and important aquaculture species in Saudi Arabia. Demand for this species has increased in spite of its darkish gray coloration, which is often considered an unattractive attribute by consumers (Pillay 1990; Lee and Newman 1992; Avault 1996; Egna and Boyd 1997).

A number of biological factors have made the various species of tilapia prime candidates for aquaculture development (El-Sayed et al. 1991; Avault 1996; Egna and Boyd 1997):

1) Tilapia can be fed on a wide variety of natural feeds in extensively managed

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[1] Tilapia is a generic term used to describe fish from the family Cichlidae, Genera *Oreochromis*, *Sarotherodon* and *Tilapia* were originally described as members of the single genus *Tilapia*.

ponds (i.e., under natural conditions) including algae, zooplankton, soft green macrophytes, and invertebrates as well as utilizing poor-quality natural foods such as blue green algae (King and Garling 1983).

2) Tilapia have been amenable to intensive culture (with the addition of nutrients and water quality management) in different types of ponds, tanks, and aquaria at different population densities.

3) Tilapia have tolerated a broad range of water quality conditions. For example, they have tolerated water temperatures ranging from 10 - 40 °C for extended periods of time, but grow best in water temperatures of 27 °C (Egna and Boyd 1997). They have survived in water with dissolved oxygen concentrations as low as 1.0 mg/L (Lee and Newman 1992) and have been raised in fresh, brackish and sea water.

4) Tilapia readily utilizes artificial feeds, and compared to many cultured fish, grow rapidly on low protein feeds.

5) Tilapia breed reliably in captivity (Lee and Newman 1992).

6) Tilapia have exhibited a high level of disease resistance and have been handled with a minimum of stress.

Some biological constraints associated with tilapia aquaculture have also been observed. The main constraint to intensive aquaculture of tilapia has been their early sexual maturation and frequent breeding. Tilapia have been shown to breed monthly when they reach sexual maturity (Lee and Newman 1992). Fry from unintended natural production after stocking has had a negative effect on growth under culture conditions because of the increased competition for feed and oxygen. Furthermore, unintended fry have complicated the management of culture ponds by making it difficult to maintain accurate inventories, project growth rates, and control feeding.

Many researchers have suggested producing all-male populations to avoid overpopulation of tilapia in aquaculture and to increase production since male tilapia grow faster and attain a larger size than females when stocks are mixed (Pillay 1990; Ridha and Lone 1990; Avault 1996). All-male stocks have been produced by the following: 1) manual sexing (Egna and Boyd 1997), 2) using of androgenic hormones to sex reverse females (Hickling 1968; Semakula and Makora 1968; Shell 1968; Ridha and Lone 1990; Phelps et al.1992; Phelps et al. 1995), 3) inducing triploids (Varadaraj and Pandian 1990; Hussain et al. 1995), 4) producing YY-supermale (Scott et al. 1989), and 5) hybridization (Lagler and Steinmentz 1957; Hickling 1960; Al-Daham 1970).

Other methods to reduce reproduction have included combined stocking of tilapia with piscivorous predators (Swingle 1950, 1960; Bardach et al. 1972; Dunseth and Bayne 1978; Wohlfarth and Hulata 1983; Ofori 1988), cage culture (Pagan 1969; Siraj et al. 1988; Lovshin and Ibrahim 1988; Mair et al. 1995), and high intensive culture systems (Swingle 1960; Avault 1996).

This experiment was designed to determine the effects of gender on performance and growth of the Nile tilapia, *Oreochromis niloticus*, in mixed and separate gender stocks using a saturation kinetics model developed by Morgen et al. (1975). The model was based on enzyme kinetics equations developed by Michaelis and Menten (1913) and Hill (1911). The general equation was based on physiological responses to different environmental factors. The fundamental basis of this model was the interaction between an independent variable, nutrient intake (I), and a dependent variable, the observed response (r), as described by the equation:

$$r = \frac{b\,(K_{0.5})^n + R_{max}\,I^n}{(K_{0.5})^n + I^n}$$

Where:

r = observed response of the fish (body weight gain or nutrient deposition at specific intake level per day),

b = ordinate intercept,

$K_{0.5}$ = nutrient constant (nutrient at ½ of R_{max}),

R_{max} = maximum response,

n = slope factor (compared to $K_{0.5}$, apparent kinetic order of the response with respect to I as I^n becomes negligible),

I = nutrient intake.

The model has been experimentally tested to measure net nutrient deposition, plasma nutrient levels, weight gain, tissue enzyme kinetics, dietary requirements and other physiological processes for many animals such as rats, mice, chickens, turkeys, fish and humans (Mercer, 1980, 1992; Belal, 1987; Belal et al. 1992; Mercer et al. 1996). This, or similar models, have been used to determine optimum feeding levels, standard metabolic rate, protein and energy requirements, and growth response for tilapia fed upon natural and artificial feeds. Because of inexpensive equipment and a relatively short time period required, this model could be used in most developing countries to determine the nutritional responses of fish to feeds. In addition, this model could be used to determine basic bioenergetic requirements of fish and to predict the production response of fish to a specific diet.

LITRATURE REVIEW

More than 70 species and sub-species of tilapia have been used or are potential candidates for aquaculture Egna and Boyd (1997). Chimits (1955) reported that both the genus of *Tilapia* and *Oreochromis* had about 100 known species, which originated in Africa and extended north to Jordan. Based on meristic, ethological, and morphological characteristics, Trewavas (1966, 1973, 1980, 1982a,b) divided the genus *Tilapia sensu latol* into three genera *Tilapia*, *Sarotherodon*, and *Oreochromis* based on parental care extended to eggs and larvae. All three genera initially spawned eggs in nests. Species of the genus *Tilapia* were defined as those species that continued parental care of their offspring in nests, while the genus *Sarotherodon* were paternal or bi-parental mouthbrooders, and the genus *Oreochromis* were maternal mouthbrooders (Pouyaud and Agnese 1995). Pouyaud and Agnese (1995) concluded that the *Tilapia senso stricto* had the largest number of ancestral characters, which confirmed the hypothesis that mouthbrooders (*Oreochromis* and *Sarotherodon*) arose from substrate spawners.

Early sexual maturation is a physiological characteristic of tilapia. Lee and Newman (1992) and Avault (1996) observed that tilapia reached sexual maturity as early as two months of age or at a length of 6 cm (2.4 inches). Mature tilapia commonly spawn from early spring until late fall and the female can spawn repeatedly during one spawning season. The optimum temperature for breeding is in the range of 24 to 29 °C (Lee and Newman, 1992; Egna and Boyd 1997).

Frequency of spawning and the age and size of the female have been identified as limiting factors that contribute to the number of eggs produced. These characteristics have created problems for commercial tilapia farmers because they reduce growth and feed conversion and it is impossible to keep an accurate inventory to calculate appropriate rates.

Many researchers have developed methods to control and manage the problems of early sexual maturation. Because male tilapia may grow faster than the female (Egna and Boyd 1997, Siddiqui et al. 1997), many researchers have developed techniques to produce all-male populations. Abucay and Mair (1997) suggested that all-male tilapia culture was important for more uniformly sized fish and higher yields. Preventing overpopulation in tilapia culture could be achieved by manually sorting the males and the females based on secondary sex characteristics, sex reversal, androgenic hormones, hybridization, stocking tilapia at high densities, stocking tilapia with natural predators, by induction of polyploidy, and by production of supermales (YY).

Manual sorting is one of the simplest ways to segregate males from females (Egna and Boyd 1997). However, there are some disadvantages to this method. First, only large and mature fish can be accurately sexed because it is hard to distinguish genders in young fish that are not ready for spawning. Moreover, keeping only the males and discarding the females result in using only approximately half of the fish. The fish also suffer from stress because of the handling process. For large-scale production, this technique has not only been time consuming but also required experienced laborers, which increased the production budget.

Successful sex reversal techniques have been developed for tilapia using various androgenic hormones (Camerino and Sciaky 1975). Sex reversal techniques have been practiced for many decades. Females have been converted to functional phenotypic males. Androgenic hormones have been applied either by injection or by oral administration in the diet. There are many factors that can impact the application of androgen hormone treatments such as fish species, fish age at administration, type and dosage of hormones and duration of treatment (Hickling 1968; Semakula and Makora 1968; Shell 1968; Guerrero 1975; Lovshin et al. 1990; Ridha and Lone 1990; Phelps et al. 1992, 1995). There have been, however, some major practical disadvantages for fish farmers. Injection of the androgenic hormones has caused handling stress to the fish. The process was labor intensive and required experienced workers. Sex reversal using androgenic hormones has not been 100% effective. The high cost of hormones and labor may not be affordable for many farmers, especially in developing countries. Furthermore, hormone treated feeds may not be effective when applied in the earthen ponds or lagoons where there are natural food sources. Thus, in order to insure that fry have consumed the hormone treated feed, tilapia should be fed treated feeds in tanks, not in ponds. However, Buddle (1984), Phelps and Cerezo (1992), and Phelps et al. (1995) reported that the efficiency of the androgen treatment was not affected in the presence of the natural feed. Androgen treatment has resulted in anatomic and morphological deformities in fish. It has caused deformities of the jaws and mouth, head and gonad abnormalities, exposed gills, short operculum, and liver malformations (Clemens and Inslee 1968; Pandian and Varadaraj 1987; Ridha and Lone 1990). However, Egna and Boyd (1997) did not observe any gross abnormalities of the fish in any of their studies. Finally, hormone treatments may also be of concern to consumers. Anabolic hormones are controlled substances and have not been approved for use in fish feeds in the U.S.A. Currently, an Investigational New Animal Drug (INAD) permit has been issued to Auburn University by the Federal Drug Administration for efficacy and safety testing of these hormones for sex reversal in tilapia with the ultimate goal of approving them for this use in fish feeds.

Thorgaard (1983) reported that sterile triploid fish exhibited faster growth than diploid fish. However Brämick et al. (1995) observed no differences in growth between triploid and diploid Nile tilapia until they reached the age of maturation. Pandian and Varadaraj (1988) found that the ovaries of triploid female tilapia were markedly underdeveloped while the size of the testes of male triploids were not affected. Pandian and Varadaraj (1988) observed that the female triploid tilapia grew 14% faster than the sex-reversed male and 23% faster than the male triploid. Brämick et al. (1995) observed that after 285 days the average body weight of both triploid Nile tilapia males and females were significantly greater than diploids of the same sex. Brämick et al. (1995) recommended raising triploid Nile tilapia whether these fish were males or females. Others, such as Mair et al. (1995) have concluded that cultivation of polyploidy Nile tilapia males rather than mixed-sex fish could increase production by 34%. In general, most studies agreed that mono-sex stocks had a higher potential yield production than mixed-sex stocks.

Varadaraj and Pandian (1989), Purdom (1993), Tave (1993), Egna and Boyd (1997) have described the procedures for supermale (YY) production. Sexually undifferentiated fry were fed a diet containing estrogen hormone to produce sex-reversed females. Sex –reversed females are phenotypic females that are genetic males. The offspring produced by feeding estrogen treated diets were a mix of normal males (XY), normal (XX) females, and sex-reversed (XY) females. The sex-reversed females were separated from the normal female genotype by analyzing the sex ratio of their offspring. Crossing a normal male with a normal female resulted in an approximately 50:50 sex ratio of phenotypic males to females. Crossing a normal male with a sex-reversed female produced an approximately 75:25 ratio of phenotypic males to females (50 XY: 25 YY: 25 XX). Supermales and normal male were identified by analyzing their offspring when crossed with normal females. Although the process has been successfully demonstrated, it required a significant investment of time and resources to produce and identify sex-reversed females and supermales. The resources and time required are beyond the ability of most fish culturists especially in developing nations.

Hybridization is another method that has been used to a limited extent to effectively manage tilapia reproduction. It can be used to produce all-male tilapia (Egna and Boyd, 1997). All-male hybrids have resulted from crossing of a homogametic female and a homogametic male (Pruginin et al. 1975; Lovshin

1982). The first generation of tilapia hybrids with T. hornorum have produced all or nearly all-male populations. In general, hybrids have grown faster and larger than either parent. One of the most commonly used hybrid tilapia crosses has been between male *Tilapia hornorum* X female *T. nilotica* that produced all or nearly all-male offspring. Production of hybrid tilapia has a number of practical disadvantages. Maintaining separate stocks of "pure" species and hybrid offspring is difficult since there may be little difference in morphological characteristics between the parent stocks and their subsequent hybrid offspring, especially in later generations (Egna and Boyd, 1997). Tilapia species readily hybridize, so it has become difficult to obtain the pure species.

Tilapia have been reared at very high densities (super intensive culture systems) in tanks, ponds, or raceways to inhibit reproduction and to enhance production. Tilapia normally exhibits aggressive territorial behavior during breeding. The male defends a territory around his nest to prevent predation of the eggs or young. If males cannot defend a territory, they may not build nests and breed with females. However, crowded fish populations require special management to provide enough feed while maintaining sufficient dissolved oxygen and safe and adequate water quality.

Predaceous fish have also been used as a method to control overpopulation in tilapia production ponds (Bardach et al. 1972; Dunseth and Bayne, 1978; Popma, 1982; McGinty, 1985; Wohlfarth and Hulata, 1983; Egna and Boyd, 1997). Predator fish such as the largemouth bass (*Micropterus salmoides*), jewel fishes (*Hemichromis spp.*), Guapote tiger (*Cichlasoma* *manauense*), peacock bass (*Cichla ocellaris*), snakehead (*Ophicephalus spp.*), crappies (*Pomoxis spp.*), and bowfin (*Amia calva*) have been suggested to control the overpopulation of tilapia in the polyculture system. The predators usually consumed the eggs, fry, and fingerlings that were produced by tilapia. Predators should not be larger than the juvenile tilapia initially stocked in the pond, otherwise the tilapia will become easy prey to the predators.

This study was designed to determine the effects of tilapia gender on performance and growth in separated and mixed-sex using a saturation kinetic model developed by Morgen et al. (1975). Using enzyme kinetic equations, Hill (1911) and Michaelis and Menten (1913) developed this model. It is also based on physiological responses to different environmental and nutritional factors. The model concept is built on the interaction between a dependent variable, the observed response (r), and an independent variable, nutrient intake (I). This model was derived from complicated formulas. The basic step is that any nutrient (I) given to any animal creates a certain response (r), whether negative or positive. For example, a nutrient (I) given to an animal, a specific receptor (M) will respond to yield the complex (MI), which produces a physiological response (pr) in a proportional manner to concentration of (MI), as follows:

$$I + M \underset{K_2}{\overset{K_1}{\Longleftrightarrow}} MI \qquad \text{------------------------------ (1)}$$

$$pr = K_3\,[MI] \qquad \text{------------------------------ (2)}$$

(Where K_1, K_2 and K_3 are constants and [ ] denotes concentration).

At equilibrium, the combination of the above equations is written as follows:

$$\frac{[M]\ [I]}{[MI]} = \frac{K_2}{K_1} = KI$$

(Where KI is also a constant)

Thus, for the total receptor concentration (Mt), where [Mt] = [M] + [MI],

or

[M] = [Mt] - [MI], then:

$$\frac{[Mt - MI]\ [I]}{[MI]} = KI$$

After rearranging, it gives:

$$\frac{[MI]}{[Mt]} = \frac{[I]}{KI + [I]}$$

When all receptors are occupied, the maximal physiological responses of the system (PR_{max}) are:

$$PRmax = K_3\ [Mt] \qquad \text{———————— (3)}$$

Then:

$$\frac{pr}{PR_{max}} = \frac{[MI]}{[Mt]}$$

$$pr = \frac{PR_{max}\ [I]}{K_I + [I]} \qquad \text{———————— (4)}$$

From this equation, comes the linear model (straight-line) equation of

Michaelis-Menten (1913), Hegsted and Neff (1970), that is:

$$Y = a0 + a1\ X \qquad \text{------------------------} \quad (5)$$

There are two limitations to equation 4 that logically and practically do not match the observed responses. First, responses are found experimentally to be sigmoidal, but this equation only describes a hyperbola. Adding the parameter n to the equation may solve this limitation:

$$pr = \frac{PR_{max}\ [I]^n}{K_I + [I]^n} \qquad \text{------------------------} \quad (6)$$

(Where n is the apparent kinetic order)

As n increases above 1, the curve of this equation fluctuates from a hyperbola to a sigmoidal ($n > 1$). This equation is known as the " Hill equation" which has been utilized in enzyme kinetics (Hill 1911).

The second limitation to this equation is in regard to the (X, Y) coordinate system, which describes a curve that passes through the point of origin (0, 0) and does not predict responses such as weight loss. To solve this, it is necessary to add the parameter b (the intercept on the ordinate y-axis) to the previous equation:

$$pr = \frac{PR_{max}\ [I]^n}{k_I + [I]^n} + b \qquad \text{------------------------} \quad (7)$$

Then:

$$pr = \frac{PR_{max}\ [I]^n + b\ k_1 + bI^n}{k_I + [I]^n} \qquad \text{------------------------} \quad (8)$$

If $(PR_{max} + b) = R_{max}$, and simplify pr to r, the four-parameter mathematical model for physiology response could be then:

$$r = \frac{b\,k_I + R_{max}\,[I]^n}{k_I + [I]^n} \qquad \text{------------------------ (9)}$$

This may be written:

$$r = \frac{b(K_{0.5})^n + R_{max}\,[I]^n}{[K_{0.5}]^n + [I]^n} \qquad \text{------------------------ (10)}$$

where:

r = Physiological observed responses in the fish,

b = intercept on the ordinate y - axis,

$K_{0.5}$ = intake constant for ½ of R_{max},

R_{max} = maximum responses,

n = slope factor (compared to $K_{0.5}$, apparent kinetic order of the response with respect to I as I^n becomes negligible),

I = nutrient intake.

This model has been used as a successful test tool with different animals such as mice, rats, turkeys, chickens, fish and humans (Mercer 1980; Belal 1987; Mercer et al. 1989; Belal et al. 1992; Mercer 1992; Mercer et al. 1993, 1996). It was used to estimate weight gain, nutrient deposition, dietary requirements, plasma nutrient levels, net nutrients, tissue enzyme kinetics and other physiological processes. Belal et al. (1992) studied two practical feeds fed to *Oreochromis niloticus* fingerlings by applying the saturation kinetic model.

Including this model, there are other models that have been used for the determination of the standard metabolic rate, optimum feeding levels, growth responses and protein energy requirements for tilapia fed both natural and practical feeds. Annett (1985) used a similar threshold-corrected hyperbolic model to demonstrate the relationship of the specific growth rate of Tilapia zillii to three different feeds. He also used this model to determine the relationship between the fish growth to fish size and water temperature.

METHODS AND MATERIALS

This study consisted of three experiments to determine the effects of gender on growth and performance using the saturation kinetics model. Male, female, and mixed-genders groups, respectively, were studied in separate feeding trials. All three feeding trials were conducted after an initial acclimation period at the Aquaculture Laboratory, Department of Fisheries and Wildlife, at Michigan State University. The female-gender feeding trial was conducted followed by concurrent feeding trials for the male and mixed-gender groups. Each feeding trial took six weeks.

Ten-gallon glass aquariums were used as the experimental unit. Six fish were stocked in each aquaria with three replicated tanks for each feeding level for a one week acclimation period. All fish were fed to satiation twice each day during the acclimation period. After the one week acclimation period, the six fish in each aquaria were weighed as a group to determine the initial weight. Fish were weighed every two weeks in order to adjust feeding rates until the end of the feeding trial. Aquaria were cleaned on a daily basis throughout each feeding trial. On the last day of each feeding trial experiment, fish from each aquarium were weighed, to determine final weight, and were euthanized using Tricaine methane sulfonate (MS-222). Fish were ground and stored frozen for subsequent analysis.

FISH

Oreochromis niloticus fingerlings were used as the experimental fish. They were obtained from Illinois State University at Normal. Fish were held at the Aquaculture Laboratory, Michigan State University for at least two weeks in order to acclimate to their new environment. During that time, fish were sexed based on primary characteristics by squeezing the ventral side of the fish, just anterior to the urogenital duct to test for gamete production and separated into three gender groups: (1) males, (2) females, and (3) unidentified sex (no gametes were observed).

Six fingerlings, 7.5 – 9.0 cm (3.0 - 3.5 inch), were randomly assigned to each tank. Fish for each gender group were randomly selected from holding tanks containing male or female fish. Each tank was covered with plastic mesh to prevent them from jumping out of the tanks. Fish were fed at satiation level twice a day for a one week acclimation period. After the acclimation period, fish were weighed as a group to determine initial weight. An additional group of 12 fish were euthanized using Tricaine methane sulfonate (MS-222), ground, and stored frozen for subsequent proximate analysis.

FEED AND FEEDING

A fresh commercial trout diet (PURINA lot number 5106), containing protein not less than 40% and fat not less than 10%, was fed throughout the feeding trials. Feed was ground to the proper size for the fingerlings then kept frozen to be used for the experimental feeding trial fish groups. Feeding levels were based on the percentage of the wet body weight for the fish, and adjusted every two weeks. The feed was fed on a dry weight basis at 0.5, 1.0, 2.0, 4.0, and 6.0% of the total wet weight per tank per day. The low feeding levels (0.5 and 1.0%) were fed once per day, while the higher feeding levels (2.0, 4.0 and 6.0%) were fed half of the total amount twice per day every 8 -14 hours. Three replicates for each feeding level were used.

WATER

Well water heated to 26 ± 2 °C (77- 81 °F) and supplied by a single pass, flow through culture system. The average water exchange rates were 30.6 L/h (8.1 gal/h). Dissolved oxygen, temperature, and pH were measured in each aquarium daily which ranged from 3.75 - 6.05 mg/L, 26 ± 2 °C, and 6.3 – 6.7 respectively. Dissolved oxygen and temperature were measured using a YSI 55 meter. Ammonia concentrations were measured weekly and ranged from 0.03 - 0.045 mg/L. The pH and ammonia concentrations were measured using a Fisher Scientific, Accumet model 25 pH/ion meter and the appropriate probes and standards for calibration. Each morning, solids (feces, food and other residues) were siphoned from the bottom of the culture tanks. There was no fish mortality throughout the feeding trials.

PROXIMATE ANALYSIS

At the beginning of a feeding trial a sub-sample of 12 fish were euthanized, which were considered to be a control fish sample. At the completion of each feeding trial, the remaining fish were euthanized. MS-222 was administrated at rate of 1 g/ 4 L of water. All the fish were ground and stored frozen at the temperature of - 7.0 to – 8.0 °C (17 – 19 °F). Proximate analysis of fish and feed samples were determined using standard AOAC (1990) methods for moisture, crude protein, crude fat, total ash, and total gross energy as dry matter basis. Moisture was determined from a one g subsample at a temperature of 105 ± 1 °C (219 – 223 °F) overnight. Total ash was determined using a muffle furnace for 18 hours at a temperature 550 °C (1022 °F). A micro-Kjeldhal system (Labconco Rapid Kjeldahl System) was used to measure the crude protein. Crude fat was measured by using Ether Extraction. Finally, a Parr 1241 Bomb Calorimeter was used to determine gross energy values (Lovell 1975).

STATISTICAL ANALYSIS:

The saturation kinetic model (equation 10), which was developed by Mercer et al. (1980) was used to estimate the four parameters (R_{max}, $K_{0.5}$, n and b) and the maintenance level of five variables (growth, crude protein, crude fat, total ash, and gross energy) for the three gender groups. This model was developed on the SYTAT software CD and provided by Dr. Mercer, Department of Nutrition, University of Kentucky at Lexington, KY. Along with the above four parameters, this model also calculated other measurements. Including the degrees of freedom, the residual standard error, student t-value, and the 95% confidence interval. Using the student t-distribution, the upper 95% quantile with

11 degree of freedom (sample size - # of parameter 15 – 4 = 11) is 1.8. Any t-value for each parameter (R_{max}, $K_{0.5}$, n and b) for all the five variables of the three gender groups greater than 1.8 in absolute value would be considered to be significant.

RESULTS

Dietary and nutrient response curves were developed for different gender groups of *O. niloticus* fed varying levels of the same commercial diet for six weeks. The shape of the curve (n), the curve parameters (R_{max}, $K_{0.5}$, b), and calculated maintenance level (ML) varied between gender groups. Feeding rate, nutrient level intake (feed fed throughout the 6 week experimental period), and observed response (weight gain or net nutrient deposition) are summarized in Table 1 and Appendix Tables 1 - 4. The proximate analysis of feed and fish from each male, female and mixed-gender group of *O. niloticus* (Table 2) were used to calculate intake and response relationships for protein, fat, ash, and gross energy (Table 3). The calculated parameters for growth, protein fat, ash and gross energy are summarized in Table 4. These calculated parameters were used to generate nutrient response curves for the three gender groups. Each point on the nutrient response curves in the following sections represents an observation from an individual tank. The dotted lines represent the 95% confidence interval. All the points below the intake (I) axis are in negative balance while all the points above the I axis are in positive balance (Drapper et al. 1966). The mixed-gender group was observed to have the wider confidence range compared to the other groups. All the gender groups grew markedly better at higher feeding rates (2.0, 4.0 and 6.0%). At low feeding rates (0.5 and 1.0%), there was a depression in the growth responses.

Table 1. The initial weight, final weight, weight gain and feed fed to female, male, and mixed gender groups of *O. niloticus* over a six week experimental period. Each value represents the mean and standard deviation for three replicate tanks per feeding rate percentage.

Gender group	Feeding Rate %	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Feed Fed (g)
Female	0.5	106.8 ± 1.4	114.7 ± 2.6	7.8 ± 1.3	22.9 ± 0.8
	1.0	121.5 ± 19.0	131.5 ± 16.9	10.0 ± 5.0	51.9 ± 7.6
	2.0	110.5 ± 11.3	163.3 ± 4.9	52.8 ± 7.7	103.2 ± 2.4
	4.0	128.7 ± 7.2	232.7 ± 5.7	104.0 ± 4.1	275.6 ± 13.1
	6.0	131.5 ± 12.4	307.7 ± 6.3	176.2 ± 9.5	480.8 ± 8.9
Male	0.5	125.8 ± 6.9	128.7 ± 7.7	2.8 ± 0.8	26.3 ± 1.4
	1.0	130.0 ± 5.4	154.0 ± 8.7	24.0 ± 12.5	55.3 ± 1.8
	2.0	127.0 ± 10.9	215.0 ± 23.9	88.0 ± 17.8	130.7 ± 13.6
	4.0	108.0 ± 17.9	263.0 ± 22.8	155.0 ± 24.3	261.5 ± 16.4
	6.0	129.2 ± 10.1	334.2 ± 6.6	205.0 ± 14.8	515.8 ± 33.0
Mixed Gender	0.5	136.3 ± 3.5	137.3 ± 2.5	1.0 ± 1.0	28.6 ± 0.8
	1.0	120.2 ± 24.7	146.5 ± 36.0	26.3 ± 11.6	54.2 ± 12.0
	2.0	108.8 ± 31.2	203.5 ± 61.6	94.7 ± 35.8	110.3 ± 31.7
	4.0	121.5 ± 19.8	252.2 ± 11.6	130.7 ± 8.1	285.9 ± 38.0
	6.0	129.3 ± 6.5	301.8 ± 7.8	172.5 ± 5.2	432.3 ± 28.0

Table 2. The dietary intake and response analysis for growth, protein, fat, ash, and gross energy by female, male, and mixed gender groups of *O. niloticus* over a six week experimental period. Intake and responses are in grams for all response variables except gross energy, which is in Kcal. Each value represents the mean and standard deviation for three replicate tanks per feeding rate percentage.

Response Variable	Feeding Rate %	Female		Male		Mixed Gender	
		Intake	Response	Intake	Response	Intake	Response
Growth (g)	0.5	22.9 ± 0.8	7.8 ± 1.3	26.3 ± 1.4	2.8 ± 0.8	28.6 ± 0.8	1.0 ± 1.0
	1.0	51.9 ± 7.6	10.0 ± 5.0	55.3 ± 1.8	24.0 ± 12.5	54.2 ± 12.0	26.3 ± 11.6
	2.0	103.2 ± 2.4	52.8 ± 7.7	130.7 ± 13.6	88.0 ± 17.8	110.3 ± 31.7	94.7 ± 35.8
	4.0	275.6 ± 13.1	104.0 ± 4.1	261.5 ± 16.4	155.0 ± 24.3	285.9 ± 38.0	130.7 ± 8.1
	6.0	480.8 ± 8.9	176.2 ± 9.5	515.8 ± 33.0	205.0 ± 14.8	432.3 ± 28.0	172.5 ± 5.2
Protein (g)	0.5	10.6 ± 0.4	-0.7 ± 1.2	12.2 ± 0.6	-1.2 ± 0.4	13.3 ± 0.4	3.8 ± 0.9
	1.0	24.0 ± 3.5	2.1 ± 1.3	25.6 ± 0.8	3.2 ± 2.9	25.1 ± 5.5	8.4 ± 2.9
	2.0	47.8 ± 1.7	12.8 ± 0.7	60.5 ± 6.3	14.0 ± 6.2	51.1 ± 14.7	21.2 ± 9.2
	4.0	127.6 ± 6.1	24.5 ± 1.3	121.1 ± 15.3	26.8 ± 6.8	132.4 ± 17.6	31.1 ± 0.7
	6.0	222.6 ± 4.1	43.9 ± 5.2	238.8 ± 7.6	43.3 ± 6.0	200.2 ± 13.0	41.4 ± 5.8
Fat (g)	0.5	0.9 ± 0.05	-1.73 ± 0.2	1.0 ± 0.1	-0.9 ± 1.1	1.1 ± 0.03	-0.1 ± 0.1
	1.0	2.0 ± 0.3	0.73 ± 0.3	2.1 ± 0.1	1.4 ± 0.6	2.1 ± 0.5	4.5 ± 2.7
	2.0	4.0 ± 0.1	5.39 ± 1.2	5.0 ± 0.5	8.0 ± 2.4	4.3 ± 1.2	12.0 ± 5.2
	4.0	10.6 ± 0.5	12.51 ± 1.3	10.1 ± 1.3	13.3 ± 2.7	11.0 ± 1.5	21.5 ± 6.2
	6.0	18.6 ± 0.3	21.15 ± 0.6	19.9 ± 0.6	30.1 ± 3.8	16.7 ± 1.1	40.4 ± 1.6

Table 2. (Continued)

Response Variable	Feeding Rate %	Female		Male		Mixed Gender	
		Intake	Response	Intake	Response	Intake	Response
Ash (g)	0.5	2.0 ± 0.1	-1.1 ± 0.3	2.25 ± 0.1	-0.77 ± 0.1	2.5 ± 0.1	-0.59 ± 0.1
	1.0	4.5 ± 0.7	-0.02 ± 0.5	4.74 ± 0.2	0.63 ± 0.7	4.6 ± 1.0	1.93 ± 1.2
	2.0	8.8 ± 0.3	4.9 ± 1.7	11.21 ± 1.2	6.17 ± 2.1	9.5 ± 2.7	10.06 ± 4.7
	4.0	23.6 ± 1.1	12.7 ± 0.7	22.41 ± 2.8	13.59 ± 3.1	24.5 ± 3.2	14.54 ± 1.0
	6.0	41.2 ± 0.8	29.0 ± 4.5	44.20 ± 1.4	27.51 ± 9.2	37.1 ± 2.4	24.24 ± 1.0
Gross Energy (kcal)	0.5	102,256 ± 3,570	278,833 ± 40,301	117,097 ± 5,999	298,114 ± 19,954	127,650 ± 3,453	387,096 ± 46,397
	1.0	231,363 ± 33,795	447,305 ± 63,697	246,558 ± 7,987	549,702 ± 62,686	241,667 ± 53,373	517,965 ± 176,100
	2.0	460,228 ± 16,367	669,330 ± 31,710	583,039 ± 60,487	926,606 ± 88,103	491,867 ± 141,192	866,349 ± 336,266
	4.0	1,229,356 ± 58,451	1,090,933 ± 54,279	1,166,494 ±147,071	1,227,142±126,813	1,275,150 ±169,656	1,180,220 ± 35,132
	6.0	2,144,400 ± 39,578	1,571,614 ± 51,559	2,300,516 ±73,014	1,670,092 ± 42,591	1,928,337 ±125,003	1,533,571 ± 68,106

Table 3. The proximate analysis of female, male, and mixed gender groups of *O. niloticus* fed varying rates of feed over a six week experimental period. Each value represents the mean and standard deviation for three replicate tanks per feeding rate percentage. All percentages are on a dry matter basis.

Gender group	Feeding Rate %	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Gross Energy (Kcal/g)
Feed		46.3 ± 1.2	3.9 ± 0.4	8.6 ± 0.7		4.5
Female	Initial	56.8 ± 0.6	8.6 ± 0.5	18.4 ± 0.8	75.1 ± 0.4	1.7 ± 0.4
	0.5	53.5 ± 1.3	2.5 ± 0.9	14.8 ± 0.2	76.2 ± 1.3	1.2 ± 0.03
	1.0	56.3 ± 0.3	9.9 ± 0.4	16.8 ± 0.4	73.6 ± 0.8	1.7 ± 0.6
	2.0	57.1 ± 0.3	15.9 ± 2.9	20.5 ± 3.1	69.9 ± 0.4	2.4 ± 0.05
	4.0	59.6 ± 1.1	21.4 ± 1.8	26.3 ± 0.9	69.0 ± 0.4	3.2 ± 0.9
	6.0	62.5 ± 1.3	24.0 ± 0.5	35.2 ± 2.9	67.4 ± 1.3	4.7 ± 1.0
Male	Initial	57.84	8.59	18.4	75.1 ± 0.4	1.7 ± 0.4
	0.5	55.3 ± 0.6	6.0 ± 3.1	16.4 ± 0.1	74.9 ± 0.7	1.2 ± 0.02
	1.0	56.6 ± 0.5	10.7 ± 1.0	17.1 ± 0.5	73.8 ± 1.8	1.9 ± 0.2
	2.0	57.6 ± 0.4	18.8 ± 0.7	21.4 ± 1.2	73.3 ± 3.1	2.9 ± 0.5
	4.0	58.8 ± 0.8	21.4 ± 1.0	25.6 ± 1.3	72.1 ± 2.7	3.9 ± 0.6
	6.0	60.4 ± 0.3	31.8 ± 2.8	32.2 ± 7.0	68.9 ± 2.1	5.7 ± 0.5
Mixed Gender	Initial					1.7 ± 0.4
	0.5	59.2 ± 3.5	7.0 ± 0.3	13.7 ± 0.5	74.5 ± 0.4	1.4 ± 0.1
	1.0	58.2 ± 3.2	15.9 ± 4.2	16.4 ± 1.0	72.7 ± 1.2	1.9 ± 0.6
	2.0	57.1 ± 2.3	22.7 ± 2.7	23.8 ± 5.8	70.5 ± 0.8	3.1 ±1.1
	4.0	59.2 ± 0.3	30.4 ± 7.7	24.8 ± 1.1	69.1 ± 0.1	4.2 ± 1.1
	6.0	59.7 ± 4.4	44.6 ± 3.1	30.5 ± 0.5	68.2 ± 0.7	5.4 ± 0.3

Table 4. Parameters (R_{max} = maximum theoretical response; $K_{0.5}$ = feed intake that produced half of the maximum response; n = kinetic order of the model curve; b = predicted response at % feed intake; ML = maintenance level) calculated for the theoretical response curves for growth, protein, fat, ash, and gross energy for female, male, and mixed gender groups of *O. niloticus* over a six week experimental period.

Response Variable	Female					Male					Mixed Gender				
	R_{max}	$K_{0.5}$	n	b	ML	R_{max}	$K_{0.5}$	n	b	ML	R_{max}	$K_{0.5}$	n	b	ML
Growth (g)	202.4	229.4	2.0	6.3	——	218.8	157.0	2.1	-5.4	26.9	189.0	132.3	1.7	-5.7	16.9
Crude Protein (g)	53.6	109.9	1.7	-1.1	11.2	51.2	101.5	1.8	-1.6	14.8	48.7	79.4	1.6	2.5	——
Crude Fat (g)	23.6	7.4	1.8	-1.8	1.8	36.9	10.7	1.9	-0.8	1.4	45.5	8.2	1.8	-0.4	0.6
Total Ash (g)	36.5	25.8	2.3	-0.5	4.0	40.3	29.5	1.8	-0.8	3.3	27.6	15.5	1.7	-1.3	2.3
Gross Energy (Kcal) *	174.2	86.9 -	1.7	24.9	——	183.1	72.9	1.6	26.1	——	168.4	69.3	1.7	31.2	——

Notes: * parameters (R_{max}, $K_{0.5}$, b, and ML) have to multiply by x 10^4

—— The ML is above the lowest feeding rate (0.5%).

GROWTH

The estimated growth response using the saturation kinetics model parameters (Table 4) is presented in Figures 1 - 3. The R_{max} for growth was significantly different among the gender groups (Table 5). The male-gender group had the highest predicted growth response (R_{max} = 218.8) followed by the female-gender group (R_{max} = 202.4 g) and the mixed-gender group (R_{max} = 189 g). The $K_{0.5}$ was highest for the female-gender group ($K_{0.5}$ = 229.4 g) followed by the male-gender group ($K_{0.5}$ = 157 g) and the mixed-gender group ($K_{0.5}$ = 132.3 g). The kinetic order of the curve (n) can be used to describe the shape of the line. When n equals one (n = 1) the shape of the curve is a hyperbola. When kinetic order of the curve is greater than one (n > 1), the curve is sigmoidal. From the estimated curves for growth, all the gender groups were in a sigmoidal shape. The highest n was 2.1 in the male-gender group, followed by the female-gender group (n = 2.0) and the mixed-gender group (n = 1.7). The estimated dietary response at zero feed intake level (b) was the highest for female-gender group (b = 6.3 g) followed by the male-gender group (b = – 5.4 g) and the mixed-gender group (b = –5.7 g. The predicted maintenance level (ML) for growth for the male-gender group was 26.9 g, which was at a feeding rate of approximately 0.5% of the total weight body weight of the fish. The mixed-gender group ML for growth was predicted at 16.87 g, which was around 0.3% of feeding rate. There was no estimation of ML predicted for the female group because the predicted curve does not intersect the x-axis.

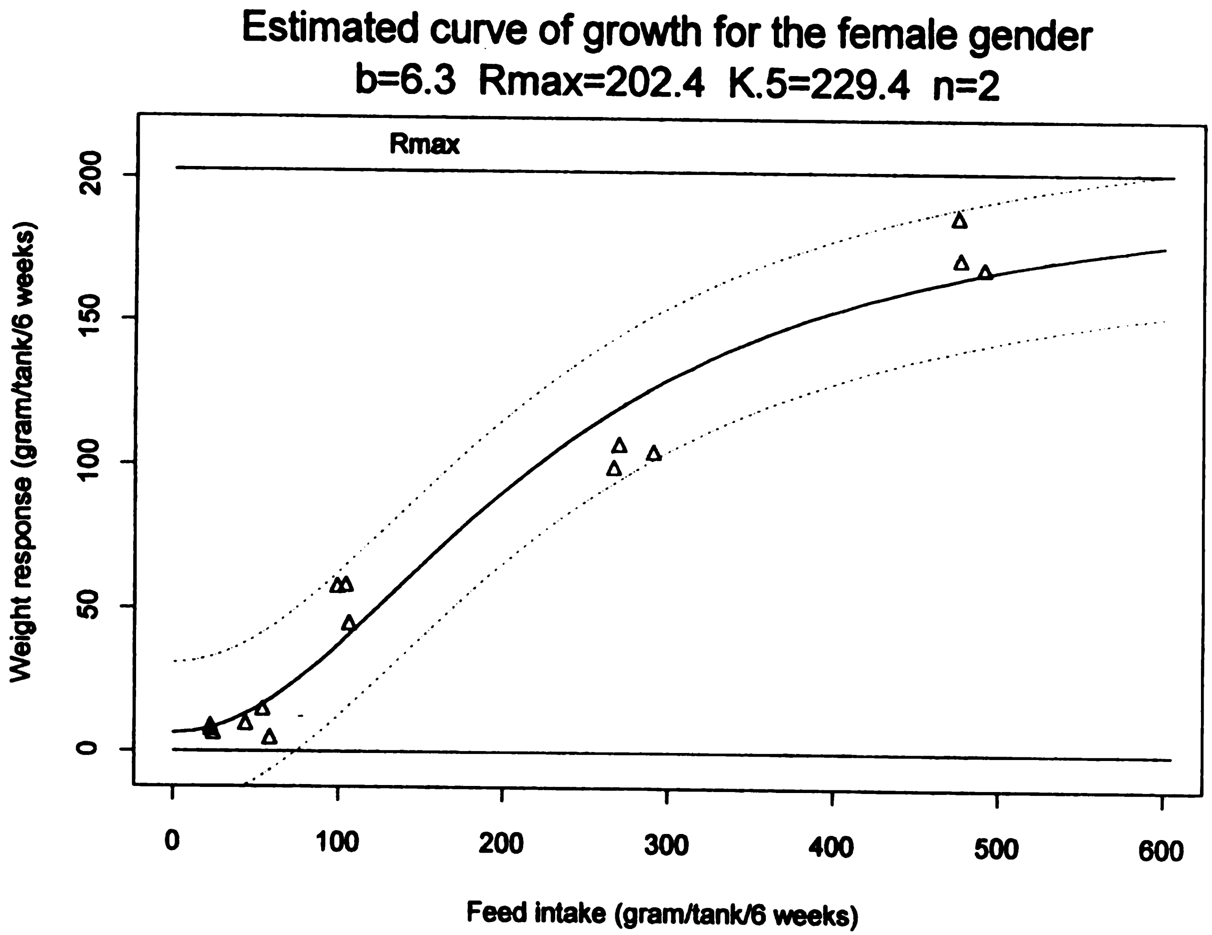

Figure 1. Theoretical nutrient curve of growth weight (g) in the feed intake vs growth weight (g) response (gain or lose) by the female of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level, and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for ach curve].

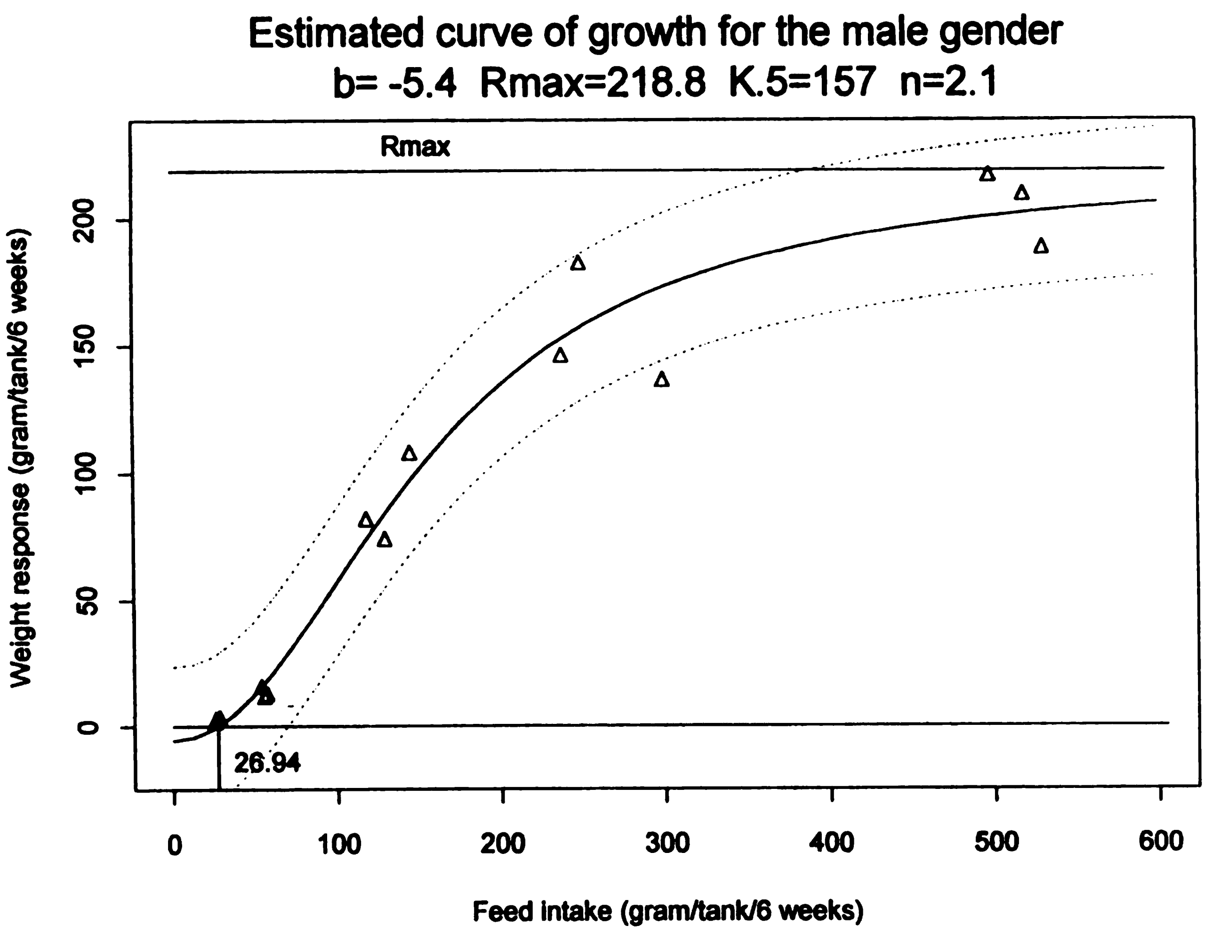

Figure 2. Theoretical nutrient curve of growth weight (g) in the feed intake vs growth weight (g) response (gain or lose) by the male of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level, and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for each curve].

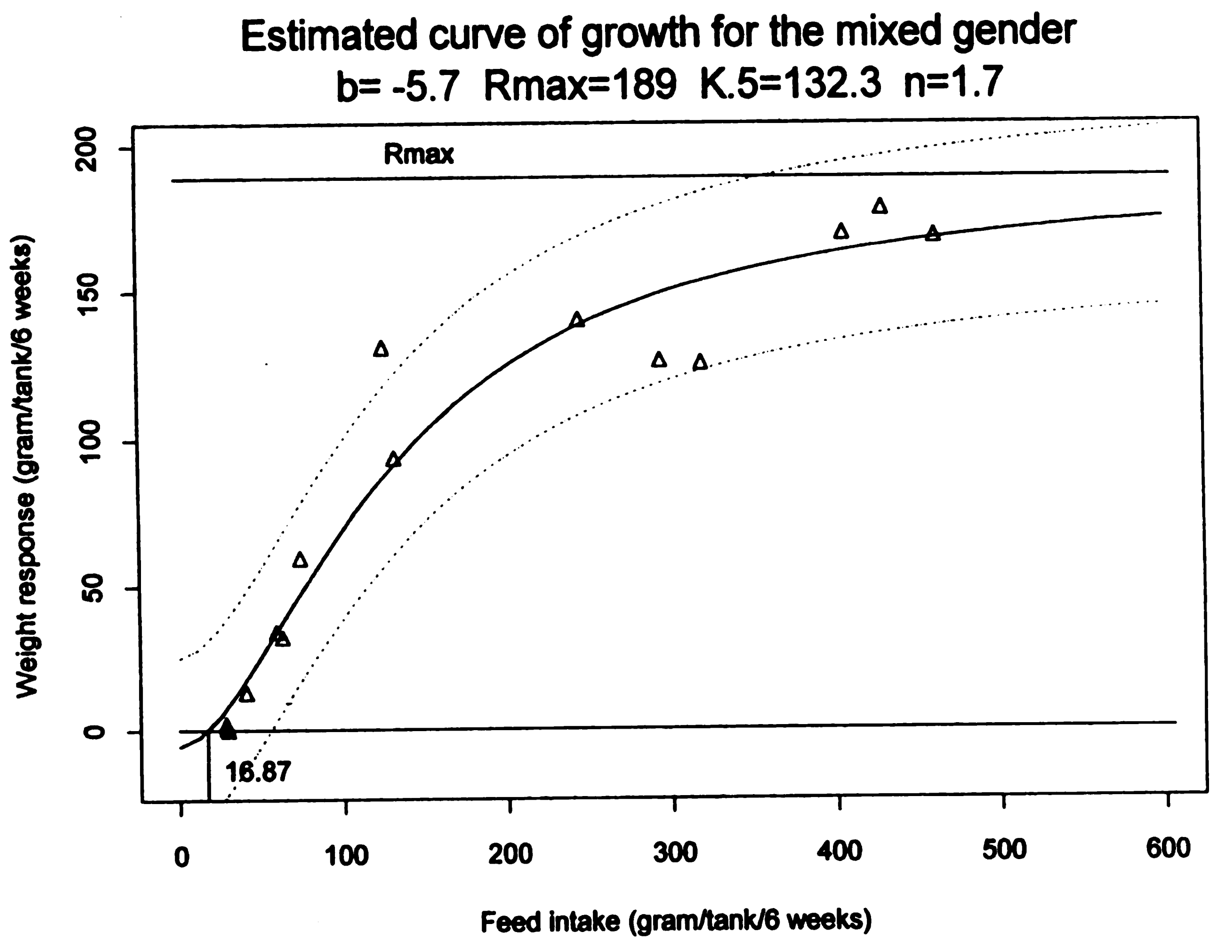

Figure 3. Theoretical nutrient curve of growth weight (g) in the feed intake vs growth weight (g) response (gain or lose) by the mixed gender of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level, and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for each curve].

Table 5: Comparison parameters table for growth between the three fish gender groups of *O. niloticus*. The degree of freedom = 11.

	Female RSE = 13.9			Male RSE = 16.2			Mixed gender RSE = 17.0		
	Parameter Value	Std. Error	t-value	Parameter Value	Std. Error	t-value	Parameter Value	Std. Error	t-value
R_{max}*	202.4	8.4	24.0	218.8	2.5	9.7	189.0	8.5	22.4
$K_{0.5}$*	229.4	5.6	14.7	157.0	0.1	7.8	132.3	2.1	11.0
n	2.0	0.2	8.2	2.1	0.6	3.4	1.7	0.2	7.7
b*	6.3	5.2	1.2	-5.4	3.2	-0.4	-5.7	7.7	-0.7
ML*	----------			26.94			16.87		

RSE: The Residual Standard Error.

*gram

**ML: Maintenance Level (g).

----: The maintenance level is above the lowest feeding rate (0.5%).

CRUDE PROTEIN

The protein response curves using the Saturation Kinetics Model parameters (Table 4) predicted are illustrated in Figures 4 - 6. The maximum theoretical amount of protein deposition in the fish bodies (R_{max}) was significantly different for all the gender groups (Table 6). The R_{max} of protein deposition for the female-gender group was 53.6 g followed by the male-gender group (R_{max} = 51.2 g) and the mixed-gender group (R_{max} = 48.7 g). The predicted $K_{0.5}$ was highest for the female-gender group ($K_{0.5}$ = 109.9 g) followed by the male-gender group ($K_{0.5}$ = 101.5 g) and the mixed-gender group ($K_{0.5}$ = 79.4 g). The kinetic order of the curve model for the protein deposition for all gender groups were sigmoidal ($n > 1$). The predicted n parameter for all the gender groups were not significantly different (male-gender n = 1.8, female-gender group n = 1.7, and mixed-gender group n = 1.6). The dietary response of protein deposition at zero feed intake level (b) varied significantly among the fish gender groups. The lowest b value was –1.6 g in the male-gender group followed by the female-gender group (b = –1.1 g) and the mixed-gender group (b = 2.5 g). The predicted ML for dietary protein level was significantly different among male and female-gender groups. The male-gender group had the highest ML of 14.8 g, which was about 0.6% of feeding rate of protein per six weeks followed by the female-gender group (ML = 11.17 g and approximately 0.47% of protein fed per six week period) and the mixed-gender group was not predicted because the ML was less than 0.

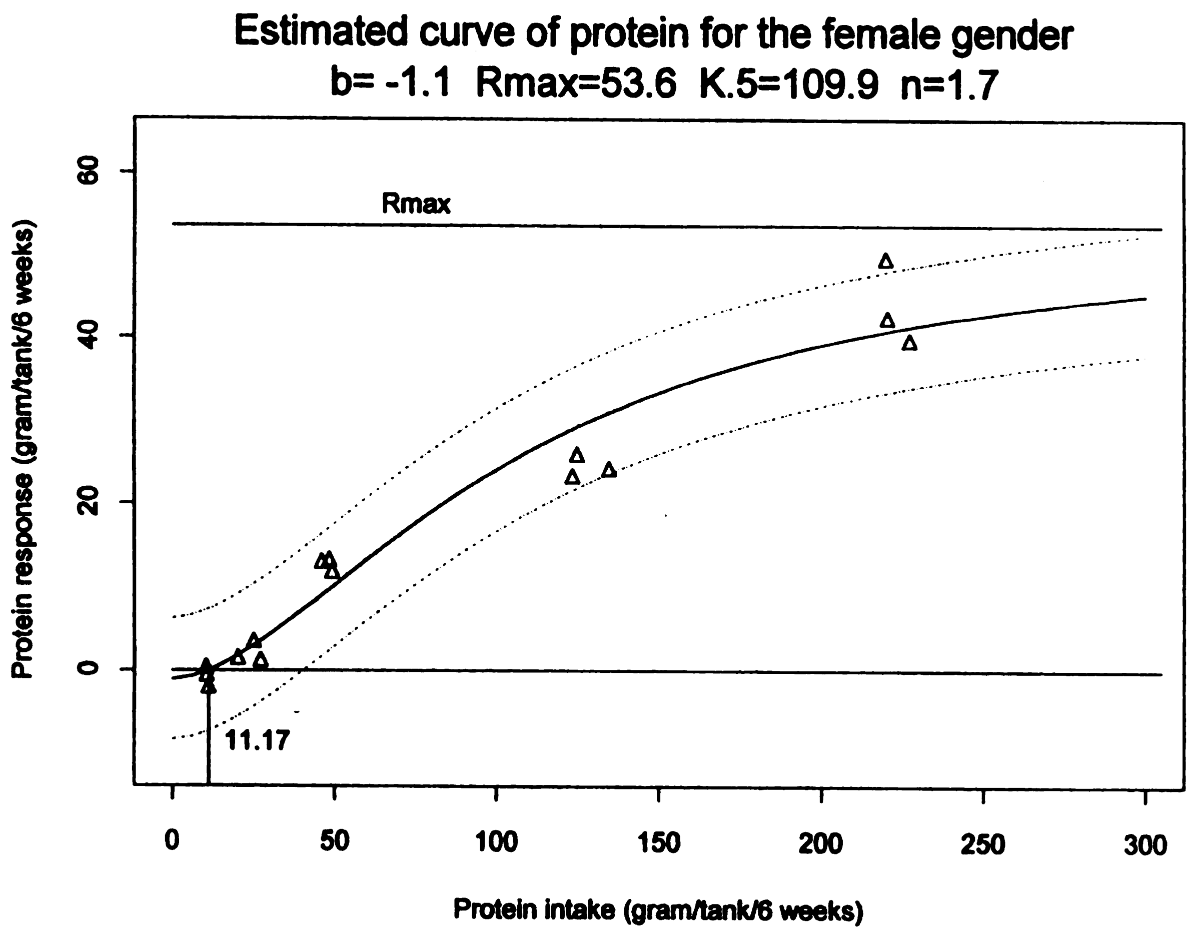

Figure 4. Theoretical nutrient curve of protein weight (g) in the feed intake vs protein weight (g) response (gain or lose) by the female of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are real the observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level, and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for each curve].

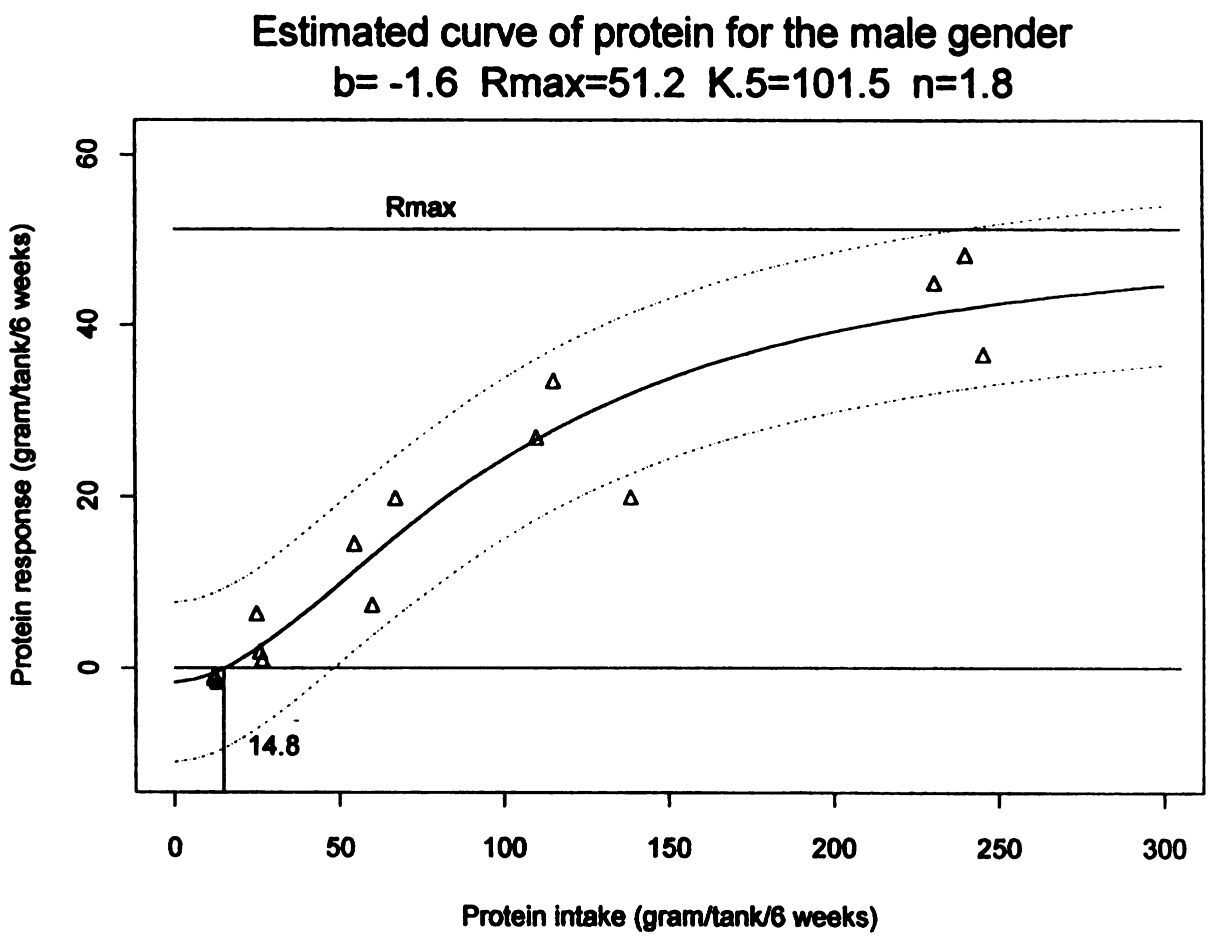

Figure 5. Theoretical nutrient curve of protein weight (g) in the feed intake vs protein weight (g) response (gain or lose) by the male of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level, and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for each curve].

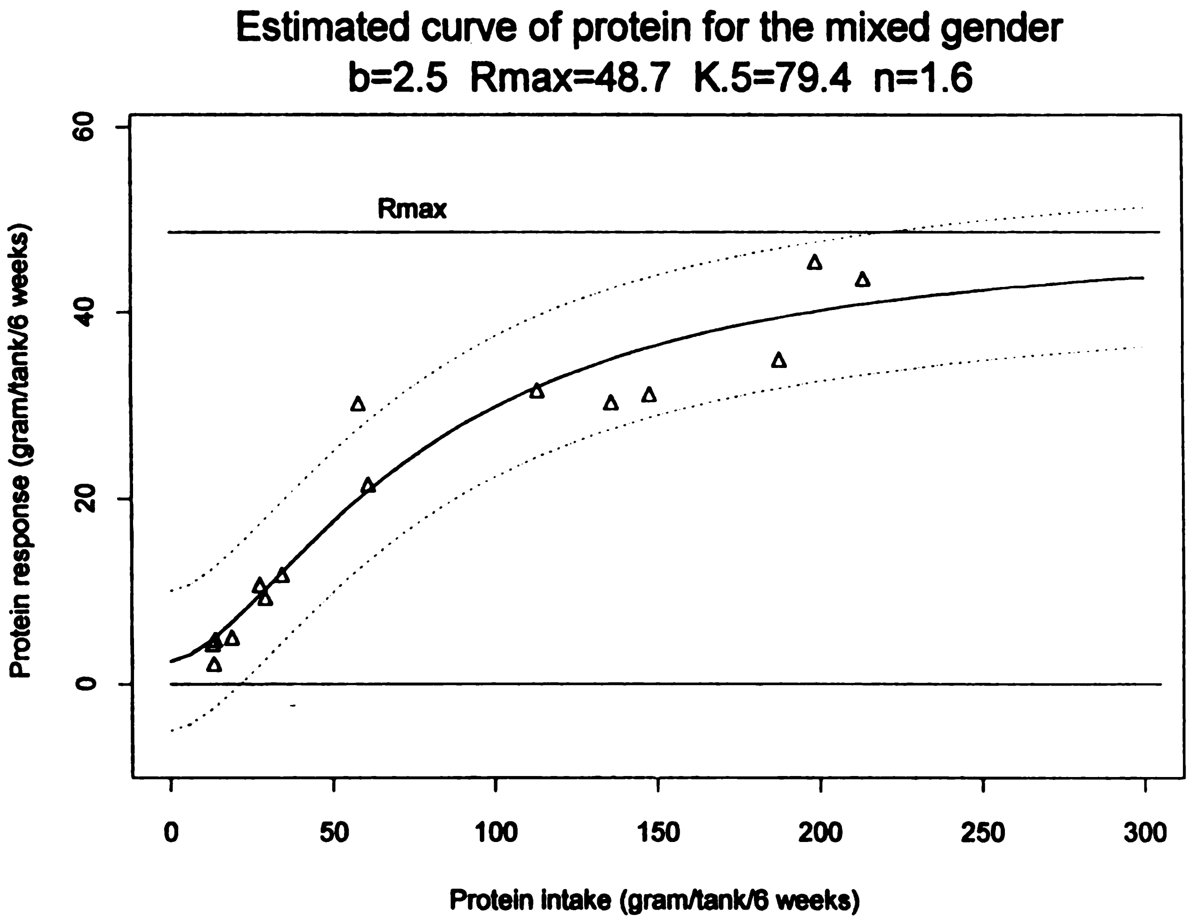

Figure 6. Theoretical nutrient curve of protein weight (g) in the feed intake vs protein weight (g) response (gain or lose) by the mixed gender of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, and the zero (0) line (parallel to the x-axes) represents the maintenance level].

Table 6: Comparison parameters table for protein between the three fish gender groups of *O. niloticus*. The degree of freedom = 11.

	Female RSE = 4.1			Male RSE = 5.2			Mixed gender RSE = 4.2		
	Parameter Value	Std. Error	t-value	Parameter Value	Std. Error	t-value	Parameter Value	Std. Error	t-value
R_{max}*	53.6	2.7	20.2	51.2	3.1	16.4	48.7	2.4	20.6
$K_{0.5}$*	109.9	8.3	13.3	101.5	9.2	11.0	79.4	7.6	10.5
n	1.7	0.2	8.1	1.8	0.3	6.2	1.6	0.2	7.4
b*	-1.1	1.6	-0.7	-1.6	2.1	-0.8	2.5	1.8	1.4
ML**	11.17			14.8			----------		

RSE: The Residual Standard Error.

* gram

** ML: Maintenance Level (g).

----: Means that the maintenance level is above the lowest feeding rate (0.5%).

CRUDE FAT

The response curves for fat deposition using the Saturation Kinetic Model (Table 4) are presented in Figures 7 - 9. The predicted R_{max} for fat deposition were significantly different for all the gender groups (Table 7). The R_{max} for the mixed-gender group was 45.5 g followed by the male-gender group (R_{max} = 36.9 g) and the female-gender group (R_{max} = 23.6 g). The $K_{0.5}$ were significantly different among all gender groups. The highest predicted $K_{0.5}$ for fat was 10.7 g for the male-gender group followed by the mixed-gender group ($K_{0.5}$ = 8.2 g) and the female-gender group ($K_{0.5}$ = 7.4 g). The kinetic order (n) for the fat deposition curve predicted that all the gender group curves were sigmoidal (n > 1) and not significantly different. Both the mixed and the female-gender groups, the n parameter was 1.8. The n value of the male-gender group was 1.9. The fat deposition responses at the zero of dietary feed intake (b) were significantly different among gender groups. The lowest b parameter was –1.8 g for the female-gender group followed by the male-gender group (b = – 0.8 g) and the mixed-gender group (b = – 0.4 g). The ML for fat deposition for all the gender groups was significantly different. The lowest predicted ML was for the mixed-gender group (ML = 0.59 g of fat per six week) followed by the male-gender group (ML =1.42 g of fat per six week) and the female-gender group (ML = 1.77 g of fat per six week).

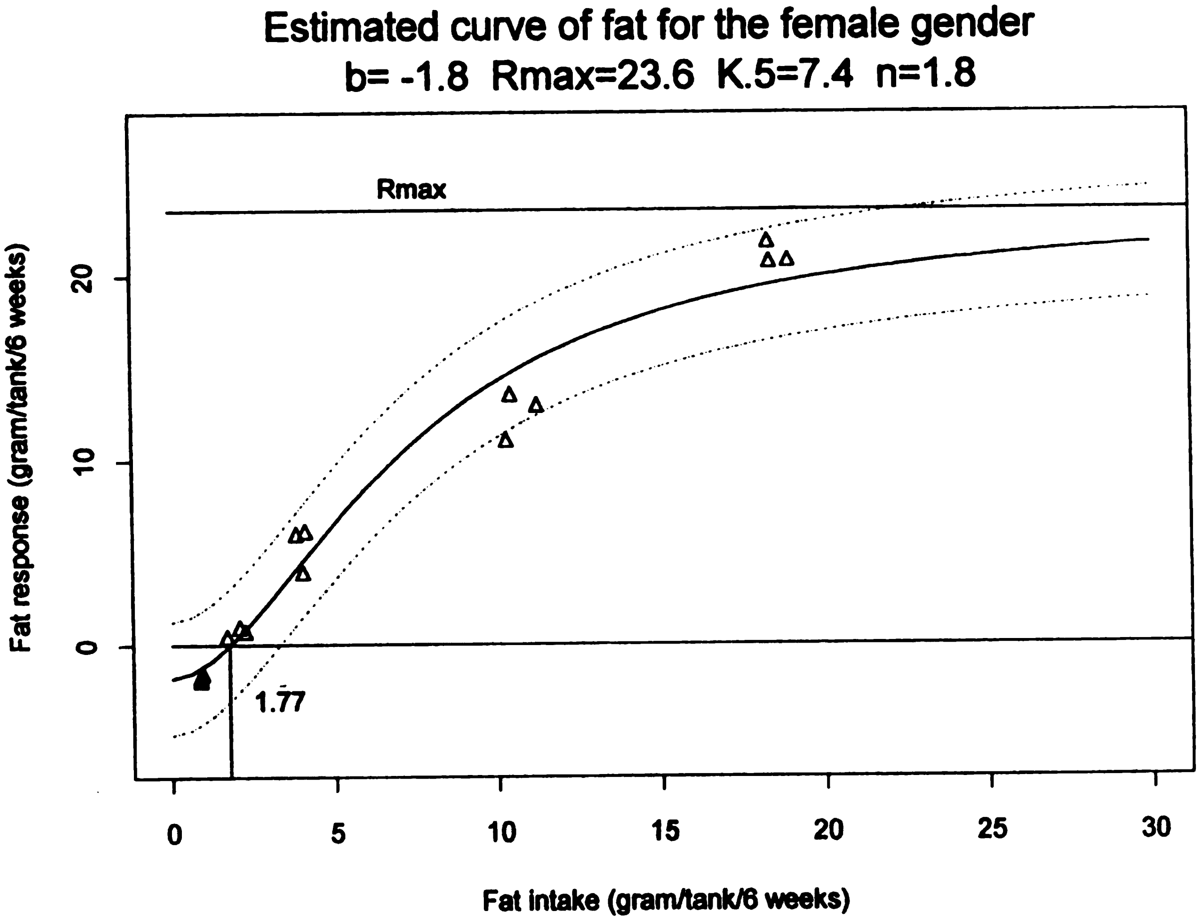

Figure 7. Theoretical nutrient curve of fat weight (g) in the feed intake vs fat weight (g) response (gain or lose) by the female of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level, and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for each curve].

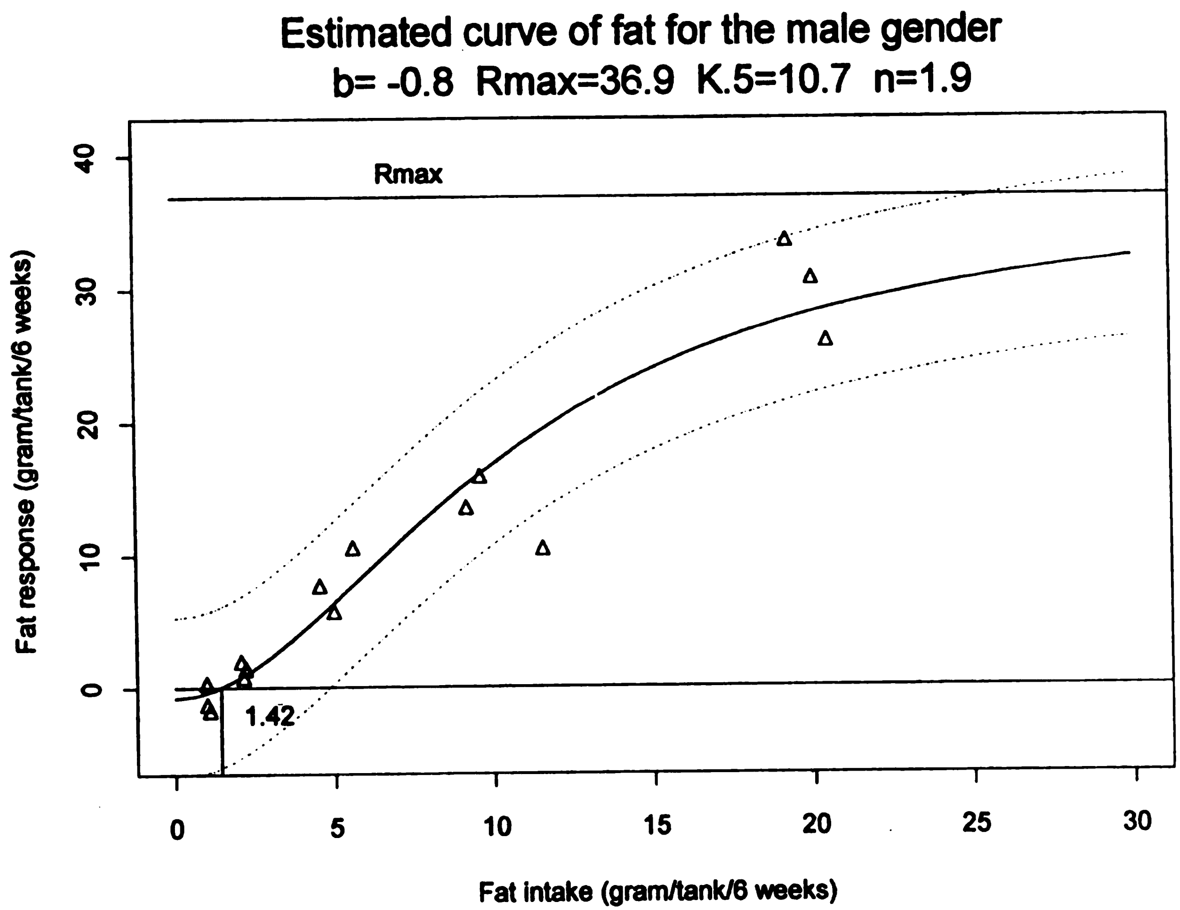

Figure 8. Theoretical nutrient curve of fat weight (g) in the feed intake vs fat weight (g) response (gain or lose) by the male of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for each curve].

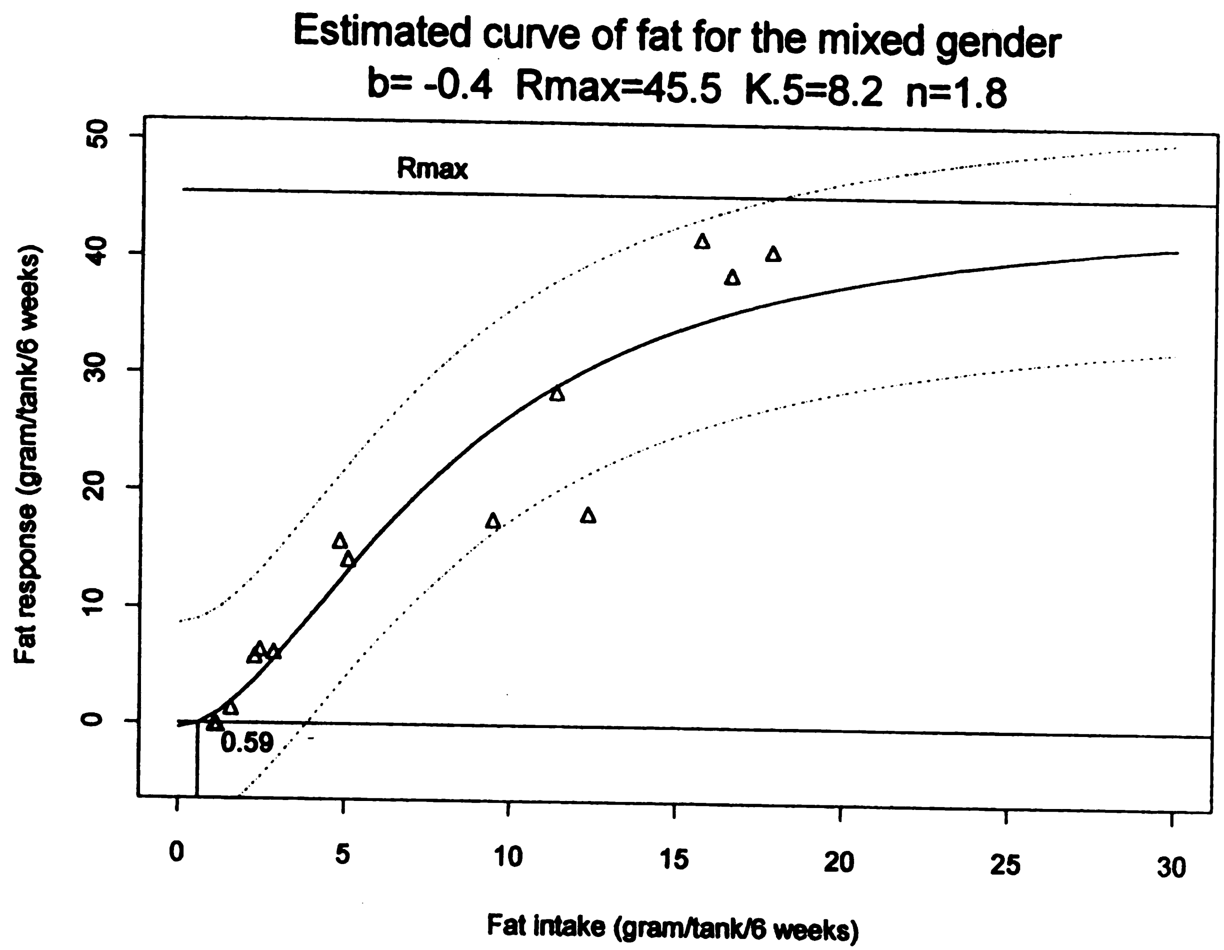

Figure 9. Theoretical nutrient curve of fat weight (g) in the feed intake vs fat weight (g) response (gain or lose) by the mixed gender of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level, and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for each curve].

Table 7: Comparison parameters table for fat between the three fish gender groups of *O. niloticus*. The degree of freedom = 11.

	Female RSE = 1.7			Male RSE = 3.4			Mixed gender RSE = 5.0		
	Parameter Value	Std. Error	t-value	Parameter Value	Std. Error	t-value	Parameter Value	Std. Error	t-value
R_{max}*	23.6	0.9	25.1	36.9	2.3	15.8	45.5	3.0	15.0
$K_{0.5}$*	7.4	0.5	15.0	10.7	0.9	12.3	8.2	0.9	9.6
n	1.8	0.2	10.0	1.9	0.3	6.3	1.8	0.3	5.7
b*	-1.8	0.7	-2.8	-0.8	1.3	-0.6	-0.4	2.0	-0.2
ML*	1.77			1.42			0.59		

RSE: The Residual Standard Error.
* gram
** ML: Maintenance Level (g).

TOTAL ASH

The estimated ash response using the Saturation Kinetic Model parameters (Table 4) for all the gender groups are presented in Figures 10 -12. The R_{max} for total whole body ash were significantly different among all gender groups (Table 8). The highest predicted R_{max} was for the male-gender group (R_{max} = 40.3 g) followed by the female-gender (R_{max} = 36.5 g of ash) and the mixed-gender group (R_{max} = 27.6 g). The predicted $K_{0.5}$ for total body ash was significantly different for all the gender groups. The highest predicted $K_{0.5}$ for total ash was for the male-gender group ($K_{0.5}$ = 29.5 g) followed by the female-gender group ($K_{0.5}$ = 25.8 g) and the mixed-gender group ($K_{0.5}$ = 15.5 g). The predicted kinetic order (n) for the total body ash predicted that all the curves for the gender groups were sigmoidal ($n > 1$). The highest predicted n was 2.3 for the female-gender group followed by the male-gender group (n = 1.8) and the mixed-gender group (n =1.7). The total ash deposition response at the zero of dietary feed intake (b) was significantly different for all gender groups. The highest predicted b parameter was – 0.5 g for the female-gender group followed by the male-gender (b = – 0.8 g) and the mixed-gender group (b = –1.3 g). The predicted ML for total ash deposition was significantly different for all the gender groups. The highest predicted ML for total ash was 4.0 g of ash per six weeks for the female-gender group followed by the male-gender group (ML = 3.34 g of total ash per six week) and the mixed-gender group (ML = 2.3 g).

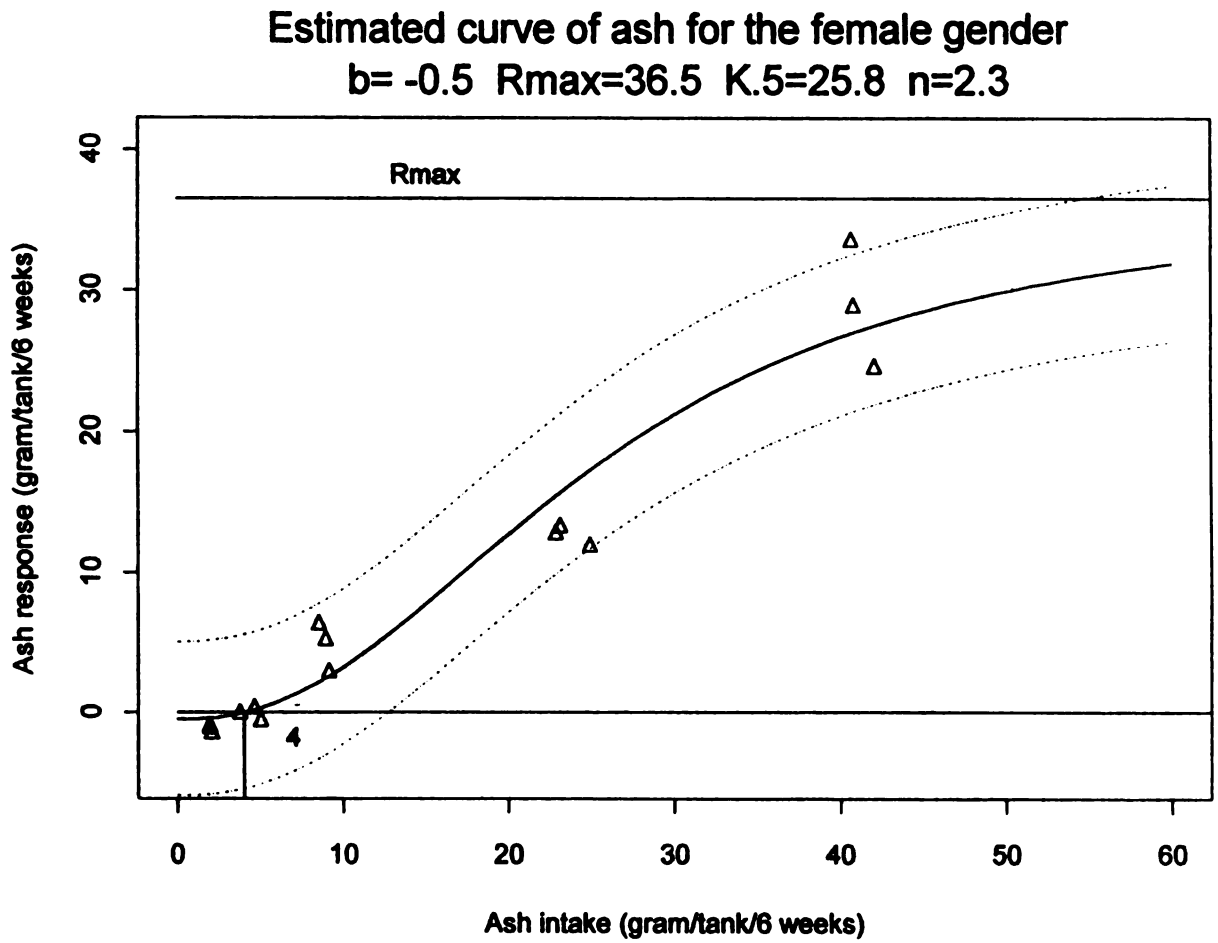

Figure 10. Theoretical nutrient curve of total ash weight (g) in the feed intake vs total ash weight (g) response (gain or lose) by the female gender of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level, and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for each curve].

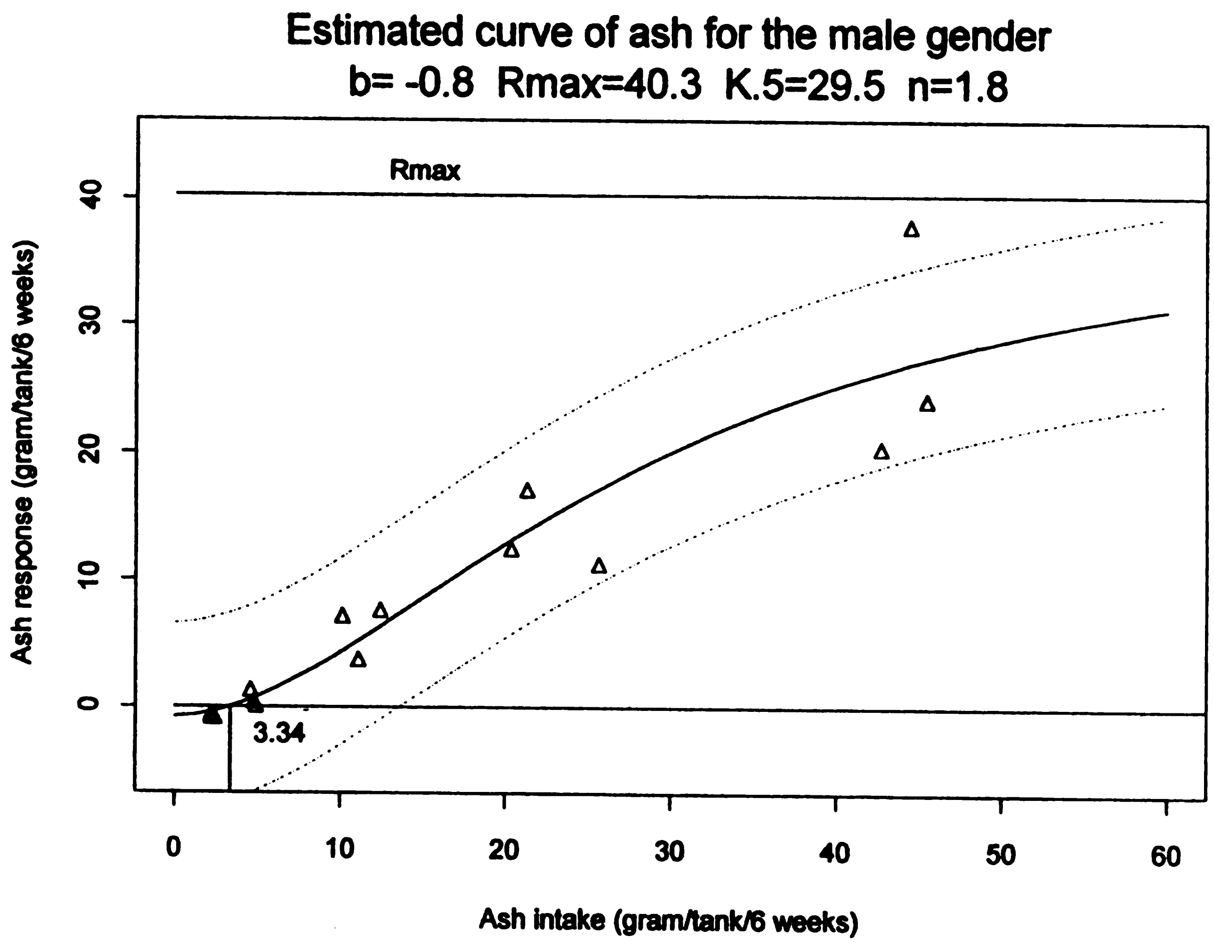

Figure 11. Theoretical nutrient curve of total ash weight (g) in the feed intake vs total ash weight (g) response (gain or lose) by the male gender of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level, and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for each curve].

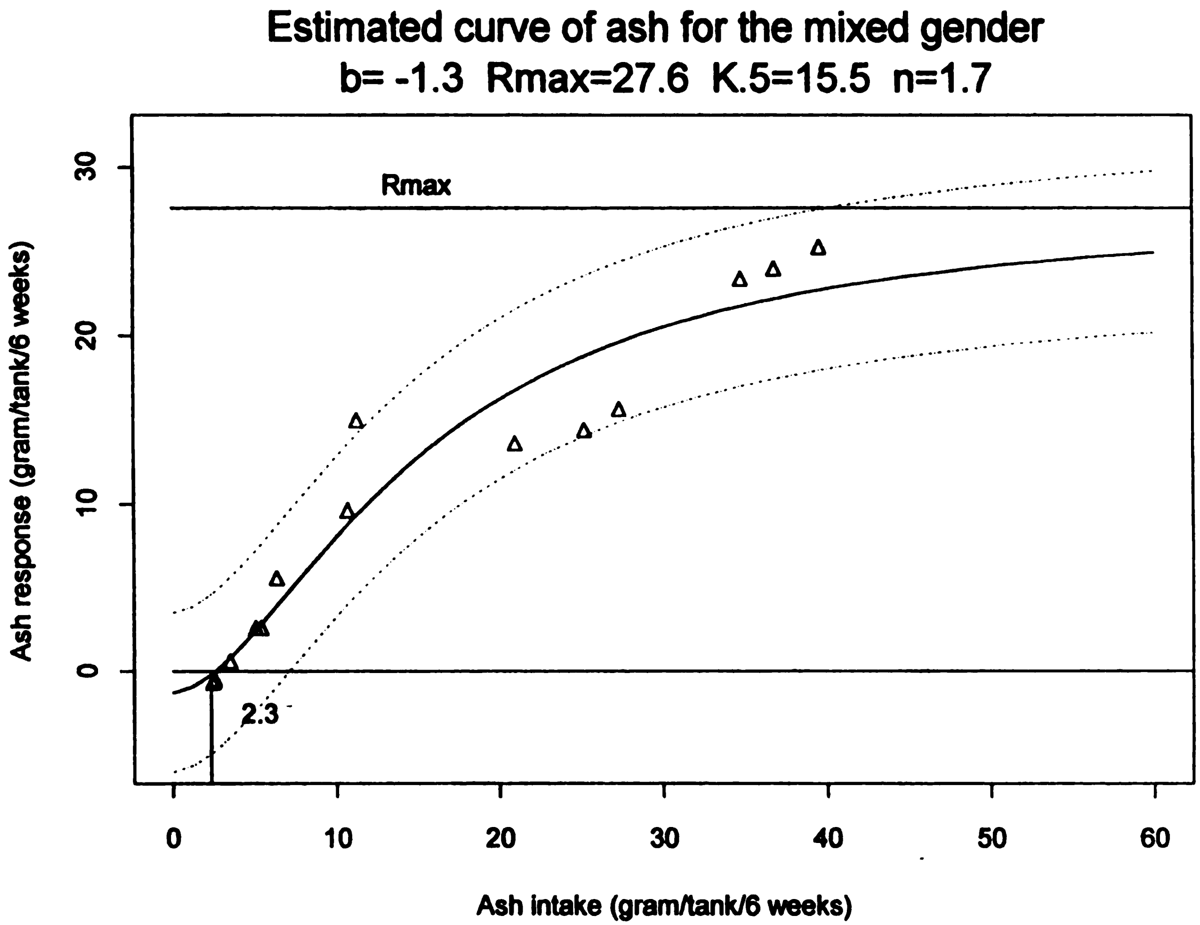

Figure 12. Theoretical nutrient curve of total ash weight (g) in the feed intake vs total ash weight (g) response (gain or lose) by the mixed gender of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval, the (Δ)s are the real observed group of fish, the zero (0) line (parallel to the x-axes) represents the maintenance level, and the perpendicular line that join both the zero line and the x-axes represents the maintenance amount of the treatment for each curve].

Table 8: Comparison parameters table for ash between the three fish gender groups of *O. niloticus*. The degree of freedom = 11.

	Female RSE = 3.1			Male RSE = 4.1			Mixed gender RSE = 2.7		
	arameter Value	Std. Error	t-value	Parameter Value	Std. Error	t-value	Parameter Value	Std. Error	t-value
R_{max}*	36.5	2.2	16.8	40.3	3.4	11.9	27.6	1.5	18.0
$K_{0.5}$*	25.8	1.7	14.9	29.5	2.8	10.4	15.5	1.5	10.5
n	2.3	0.4	5.7	1.8	0.3	5.4	1.7	0.2	7.1
b*	-0.5	1.1	-0.4	-0.8	1.5	-0.5	-1.3	1.1	-1.1
ML**	4.0			3.34			2.3		

RSE: The Residual Standard Error.

* gram

** ML: Maintenance Level (g).

GROSS ENERGY

The estimated theoretical total energy deposition response (Kcal) using the Saturation Kinetic Model (Table 4) for each gender group are presented in Figures 13 –15. The predicted R_{max} for total body energy deposition were significantly different for all the gender groups (Table 9). The highest R_{max} was 183.1 x10^4 Kcal of energy predicted for the male-gender group. The predicted R_{max} for female-gender group was 174.2 x 10^4 Kcal. The lowest R_{max} was 168.4 x 10^4 Kcal of energy for the mixed-gender group. The predicted $K_{0.5}$ was significantly different for all the gender groups. The highest $K_{0.5}$ for energy deposition was 86.9 x 10^4 Kcal predicted in the female-gender group followed by the male-gender group ($K_{0.5}$ = 72.9 x 10^4 Kcal) and the mixed-gender group ($K_{0.5}$ = 69.3 x 10^4 Kcal). The kinetic order (n) for the energy curves were not significantly different among the three gender groups. All predicted response curves for the gender groups were sigmoidal ($n > 1$). For both the female and the mixed-gender groups, the predicted n was 1.7. The estimated kinetic order (n) of the energy for the male-gender group was 1.6. The dietary response of net energy at zero feed intake level (b) was significantly different among the fish gender groups. The lowest predicted b was 24.9 x 10^4 Kcal of net energy for the female-gender group followed by the male-gender group (b = 26.1 x 10^4 Kcal) and mixed-gender group (b = 31.2 x 10^4 Kcal). The predicted ML for dietary energy for all gender groups was greater than zero (Figures 13 – 15 and Table 4).

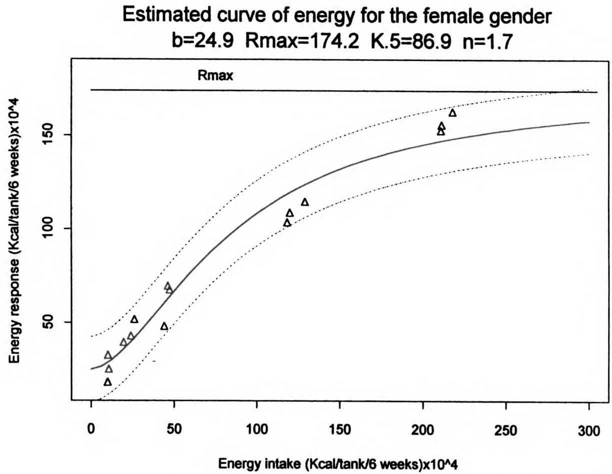

Figure 13. Theoretical nutrient curve of energy (Kcal) in the feed intake vs energy (Kcal) response by the female of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval and the (Δ)s are the real observed group of fish].

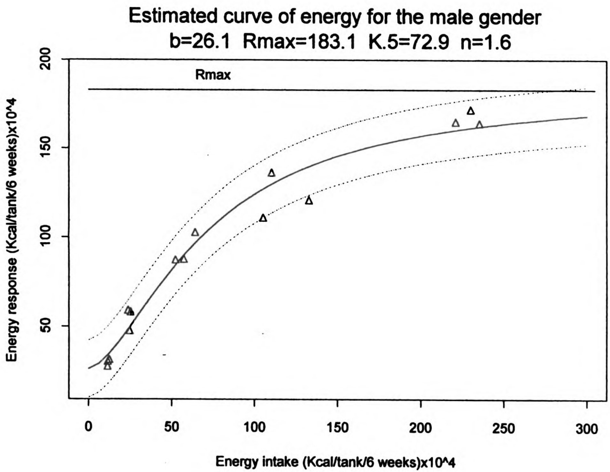

Figure 14. Theoretical nutrient curve of energy (Kcal) in the feed intake vs energy (Kcal) response by the male of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval and the (Δ)s are the real observed group of fish].

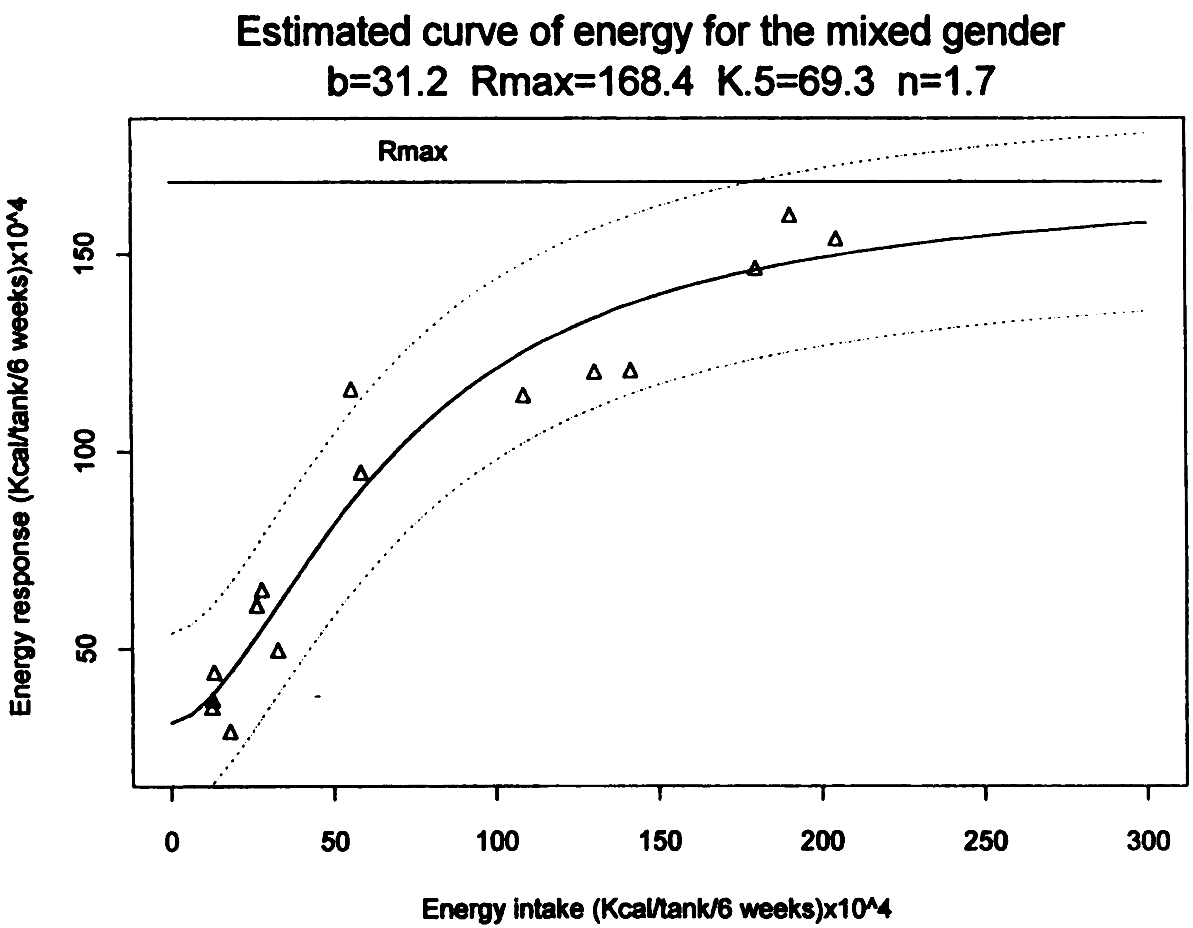

Figure 15. Theoretical nutrient curve of energy (Kcal) in the feed intake vs energy (Kcal) response by the mixed gender of Nile tilapia *O. niloticus* fish throughout the experiment period (6 Weeks). [The dotted line is the 95% confidence interval and the (Δ)s are the real observed group of fish].

Table 9: Comparison parameters table for **gross energy** between the three fish gender groups of *O. niloticus*. The degree of freedom = 11.

	Female RSE = 9.7			Male RSE = 9.0			Mixed gender RSE = 12.7		
	Parameter Value	Std. Error	t-value	Parameter Value	Std. Error	t-value	Parameter Value	Std. Error	t-value
R_{max}*	174.2	5.6	31.2	183.1	4.9	37.2	168.4	6.8	24.7
$K_{0.5}$*	86.9	5.7	15.2	72.9	4.3	17.1	69.3	6.6	10.5
N	1.7	0.2	10.1	1.6	0.1	11.2	1.7	0.2	7.5
b*	24.9	3.8	6.5	26.1	4.0	6.5	31.2	5.5	5.7
ML*	----------			----------			---------		

RSE: The Residual Standard Error.

*gram

**ML: Maintenance Level (g).

----: Means that the maintenance level is above the lowest feeding rate (0.5%).

DISCUSSION

The primary goal of this study was to use the saturation kinetic model (Morgan et al. 1975) to describe *O. niloticus* weight gain and net nutrient deposition as a function of nutrient intake by gender. The physiological response variables tested (weight gain, protein deposition, fat deposition, total ash deposition, and net energy deposition) were used to generate the saturation kinetic models (Figures 1-15 and Table 4). Responses could be calculated at any intake level using the model equation after estimating the parameters (R_{max}, $K_{0.5}$, n, and b) and ML. Each of these parameters interacts with one anther either directly or indirectly. R_{max}, $K_{0.5}$, n, and b define the response by the following equation:

$$r = \frac{b\,(K_{0.5})^n + R_{max}\,I^n}{(K_{0.5})^n + I^n}$$

The second goal of this study was to use the model parameters to determine differences in basic nutritional requirements between the gender groups of *O. niloticus*. To compare responses between gender groups, all parameters and interactions must be considered.

GROWTH

Previous studies on cultured tilapia species have indicated that growth and performance were varied between mono-sex and mixed-sex cultures. For instance, Torran and Lowell (1986) reported that the growth rates and mean weights for male and female in mixed-gender population of *Oreochromis aureus* tilapia stocked into channel catfish (*Ictalurus punctatus*) grow out ponds were 2.06 and 1.72 g / day and 249 and 211 g, respectively.

The hypothesis for growth for this study was that the intake-response curves would be equal for all gender groups (male, female, and mixed-gender). The results indicated that the responses were not equal between gender groups. In this study, growth and performance between gender groups were varied. In general, the responses by the male-gender group were superior compared to the other groups (the female and the mixed-gender groups). The maximum theoretical response for growth (R_{max}) can be used to estimate the genetic growth potential of fish relative to feed intake. As the responses approach Rmax the nutrient causing that response becomes less limiting. This will continue until limitation on the response passes to some other non-nutritional source such as the genetic potential of the fish. Similar growth responses were observed using the saturation kinetics model by Belal et al. (1992) when they compared the growth rates of O. *nilotica* fed diets from different countries.

Superior male tilapia growth rates have been reported by Van Someren and Whitehead, (1960); Fryer and Iles (1972); Kubaryk (1980); Egna and Boyd, (1997); Siddiqui et al. (1997); Abucay and Mair, (1997). The results of this study are in agreement with these previous studies. The theoretical maximum response (R_{max}) value was (218.8 g) for the male-gender group, was significantly highest among the other groups. Surprisingly, the mixed-gender group had the lowest predicted response (189.0 g). Size variance was observed for fish in the mixed-gender group. Furthermore, the response curves from our study indicated that the mixed-gender group had wider confidence limits than the other gender groups. The wider 95% confidence limits for mixed-gender group might have been the result of gender variation.

Mair et al. (1995) observed a 34% increase in male tilapia production compared to mixed-gender production. They confirmed that all-male populations resulted in higher yields of marketable fish than mixed-gender populations. Comparing the growth rates of all-male and all-female of *Tilapia nigra* populations, Van Someren and Whitehead (1960) found that the separation of sexes did not affect the faster growth of the males. This superiority of male growth is due to genetic, environmental, physiological, and behavioral reasons. Goudie et al. (1995) suggested that there is a major influence of sex on growth that must be considered to evaluate the role of the genes related to growth along with the environmental factors. Also, Fryer and Iles (1972) studied *Tilapia aurea*, and they concluded that along with the environmental factors that moderated the male growth superiority, genetic basis is a considerable factor could influence the growth.

McGinty (1985) found that the growth rates of tilapia depends on to a greater extent on the organization of the social hierarchy. The aggressive behavior and competition for food and territory probably depressed the Rmax of the mixed-gender group. The limited volume of water (as an environmental condition) in our experimental tanks (10 - gallon) may have limited reproductive behaviors and resulted in higher feed conversion ratios even though reproduction was not observed in any of the tanks.

Metabolic rate could be another factor, which contributed to increased growth response by male tilapia. Van Dam and Penning De Vries (1995) observed that the metabolic rate of *O. niloticus* was 50% higher than the catfish (*Clarias gariepinus*) because catfish were less aggressive. Hallerman et al. (1986) suggested that different male and female allozymes physiologically influenced the growth channel catfish (*Ictalurus punctatus*). This study agrees with the observations of previous studies in that the male group growth rates were higher, probably due to the higher potential metabolic rate, compared to the females whether they were raised separately or in mixed-gender groups.

The amount of feed intake that produced half of the maximum growth response ($K_{0.5}$) in gram of feed / fish tank / six weeks for all the gender groups were estimated. The optimum response is achieved with the highest possible R_{max} response combined with the lowest possible $K_{0.5}$ value. Accordingly, with consideration to the R_{max} value, the female-gender group used the highest predicted consumption (the less efficient) estimated $K_{0.5}$ = 229.4 g. Whereas, the male-gender group had the lowest significant estimated $K_{0.5}$ = 157.0 g with the highest R_{max} value among the gender groups. In contrast, the mixed-gender group had the lowest $K_{0.5}$ = 132.3 g, and R_{max} value (189.0 g) the lowest of the all groups. The differences of $K_{0.5}$ between the groups could be an indicator for the effects of the sex and metabolic rate on the feed uptake.

The predicted kinetic order for the response curve (n) was greater than one, indicating a sigmoidal relationship. Differences in the n parameter value reflect differences in the magnitude of response. The high n value is a result of high R_{max} response.

The predicted dietary responses of growth at zero feed intake level (b) varied among the gender groups. The negative sign for this parameter for males and mixed gender groups indicated fish were using nutrients from their bodies. This parameter indicated the feed fed was insufficient to meet their basal metabolic and daily activity requirements. The low b value obtained reflects the high loss of fish body weight or nutrient. This parameter is directly related to the R_{max} parameter. The highest (the best) predicted b value for the growth variable was 6.3 g for the female-gender group. There was no significant difference for the predicted b for both the male and the mixed-gender groups, which were -5.4 and -5.7 g, respectively. The high b value for the female-gender group probably indicated that they have the highest feed efficiency to utilize their feed intake at low feeding rate than the male whether separately or with the mixed-gender group. Although, the female performed best at low feeding rates, males performed better at higher feeding rates as demonstrated by differences predicted in b versus R_{max}. Significantly, the feeding levels influenced the mean final weight and feed conversion ratios (Siddiqui et al. 1997). This predicts that the female-gender group had preformed growth better than the male at the low feeding rates, while the male-gender group gained weight better than the female-gender group at the high feeding rates. Simco et al. (1989) reported that the sex of channel catfish (*Ictalurus punctatus*) did not influence the growth at a young age, but sex had an increasing influence with increasing age.

The zero (0) line of response (maintenance level) was significantly different for the growth response among the male and mixed-gender groups. The optimum ML occurred at the minimum feed intake at 0 response. In other words, the ML was the level of feed intake that met the daily activity requirement without gaining or losing weight. The lower ML (the most efficient) for the growth was predicted for the female-gender group. There was no predicted value for ML for the female-gender group because the lowest feeding rate (0.5%) provided sufficient feed to meet the ML requirements. This indicates the high efficiency of the female-gender group to maintain their growth at low level of feeding compared to the male separately or with the mixed-gender group. The ML of the mixed-gender group was 26.9 g of feed per six week, which was approximately 0.5% of feeding rate. The ML for the male-gender group was 16.9 g of feed per six week, which was approximately 0.3% of fish body weight per day. The previous reasons could be involved increasing the ML for the males, whether they were separate or mixed with the females in the mixed-gender group.

CRUDE PROTEIN

Although the predicted responses of crude protein between the gender groups were not significantly different for the R_{max} value, there were significant differences in the $K_{0.5}$. In this case, the male-gender group most efficiently utilized protein compared to the other gender groups. The ratio of dietary protein to energy is an important factor to promote growth efficiency and maximize feed conversion (Garling and Wilson, 1976). Protein has also been shown to be a major source of energy for fish (Steffens, 1989). The male-gender group was predicted to have the highest efficiency level of protein intake compared to the other groups. Thus, the male-gender group consumed more protein, which could be used as an energy source. The results of this study are in agreement with the above studies.

The estimated response of b parameter of protein intake level was significantly varied between the gender groups. The highest b parameter of protein was 2.5 g for the mixed-gender group, followed by the female-gender group (b = -1.1 g of protein) and the male-gender group (b = -1.6 g). As indicated above, the male-gender group had lower efficiency at the low feeding rates (0.5 and 1.0%), where they used more protein than the other gender groups for maintenance and daily activities. In the case of the mixed-gender group, they did not loose protein from their bodies. Predicted data indicated that the mixed-gender group gained some protein at low feeding rates during the experiment.

The estimated ML of the protein requirements varied significantly among the male and female-gender groups. The highest (worse) predicted protein requirement for ML was 14.8 g of protein for the male-gender which was predicted at 0.6% of feed per body weight of fish per day. Fish have been shown to derive ATP mainly from protein when fasting or at low feeding levels (Love 1980). This is consistent with our observations of the response of the male-gender group. The high ML value for the male-gender group may have been due to the high requirement of protein that was not adequate to meet ML at low feeding levels. The female group was more efficient than male group in using protein at low intake levels (ML = 11.2 g, at 0.5% of feed per body weight of fish per day). The mixed-gender group was the most efficient in protein utilization. The ML = could not be predicted from the predicted growth response by saturation kinetics model since it would have been less than 0.

CRUDE FAT

Fat has been shown to be a very important energy source for fish (Halver 1972). Typically, the total live weight fat content of tilapia has been between 5 and 6% (Balarin and Hatton 1979). In this study, there were significant differences between the gender groups in the predicted fat deposition response. The R_{max} differences among the gender groups were significant. The highest predicted R_{max} was 45.5 g of fat deposition per six week was observed for the mixed-gender group. The estimated R_{max} for the male group was 36.9 g of fat deposition. The lowest predicted R_{max} was 23.6 g of fat deposition for the female-gender group.

The estimated $K_{0.5}$ for fat deposition for all the gender groups were significantly different. The highest estimated $K_{0.5}$ was 10.7 g of fat deposition for the male-gender group which probably resulted from the high energy requirement for basal metabolism and daily activities as indicated previously for the growth and protein variables.

The b parameter is directly related to R_{max}. The lowest estimated b was –1.8 g for the female-gender group and indicated that the female-gender group had the lowest efficiency of crude fat utilization. The highest predicted b was -0.4 g of fat for the mixed-gender group. The predicted b for the male-gender group was -0.8 g. This explains the high R_{max} of fat deposition for the mixed-gender and depression for the both the female and male-gender groups. Socially, the aggressive behavior of males in the mixed-gender group may have caused the females to use fat from their bodies as an energy source. The predicted fat level for maintenance supports this assumption.

The highest predicted ML of crude fat requirement and fat deposition for the mixed-gender group was 0.6 g, which was predicated at the 0.3% of feed per fish weight per day. The ML for the male and the female-gender groups were 1.4 and 1.8 g for fat deposition, respectively, which are 0.7 and 1.0% of feed per fish weight per day. This indicated that the female group had the highest fat requirement (least efficient) to meet their daily needs compared to the other gender groups. Females divert energy into egg production, which reduces the amount of energy available for somatic growth (Mair et al. 1995).

TOTAL ASH

Unlike land animals, fish can meet part of their requirements for some minerals, calcium for example, directly from the water through gill uptake (Steffens 1989). Other minerals must be obtained by fish from their feed, such as phosphorus (Halver 1972). Therefore, interpreting the total ash response for fish can be difficult. The Saturation Kinetic Model predictions were based only on the total ash levels provided by feed without consideration to minerals that may have been provided by the hard water used in our experiments.

The predicted R_{max} for total ash were significantly different among the gender groups. The highest estimated R_{max} for ash deposition was 40.3 g for the male-gender group. Then the R_{max} for the female and the mixed-gender groups were 36.5 and 27.6 g, respectively. The highest R_{max} for the male-gender group was probably due to their higher and faster potential metabolic rate of growth compared to the other gender groups. The mixed-gender group had the lowest estimated b (-1.3 g) and the lowest estimated R_{max} (27.6 g) indicating that the males may not have been responding at the same level compared to the male-gender group. The estimated $K_{0.5}$ parameter for total ash also supports this conclusion. The highest predicted $K_{0.5}$ for total ash was 29.5 g for the male-gender group. This indicates that the male-gender group had the highest demand for total ash for growth among the groups.

The highest prediction for b was -0.5 g of total ash for the female-gender group because they had less demand than the male-gender group. The lowest b was –1.3 g of total ash for the mixed-gender group because they gained the lowest R_{max} of total ash. In other words, they have lowest demand of growth due to the genetic, social, and physiological factors between the male and the female in the mixed-gender group. The highest predicted ML was 4.0 g for the female-gender group. This indicates that the female-gender group has probably the highest requirement for maintenance of total ash. Also, the high ML could be used an indicator for the high demand of total ash for growth. On other hand, this indicted that the male-gender group utilized total ash more efficiently and / or retained more ash than the female group.

GROSS ENERGY

Steffens (1989) reported that energy could be stored as protein or fat in the fish body. The predicted energy deposition was significantly different among all gender groups. The higher predicted gross energy response for the male group was probably due to their capability of utilizing fat and protein compared to the other gender groups. Feed consumption has been show to be regulated by caloric intake (Lee and Putman 1973) which was supported by our observations of R_{max}. Larger fish tend to a higher rate of energy consumption per unit gain (Steffens 1989). The lowest predicted R_{max} response for gross energy was for the mixed-gender group. This was probably due to the social competition between males for females, which caused expended energy for defending territories by the males in this group. Moreover, genetic and metabolic factors for the females may have decreased the R_{max} of this group (Hallerman et al. 1986). Observations of the $K_{0.5}$ parameter support this assumption. The estimated $K_{0.5}$ for deposited energy were significantly different for all the gender groups. The highest (worse) $K_{0.5}$ for the female-gender group. The $K_{0.5}$ for both the male and the mixed-gender groups were not significantly different. The higher estimated $K_{0.5}$ value for the female-gender group indicated that they required higher feed amounts to meet the half-maximum response.

At the zero (0) feed intake level, the predicted b for gross energy were significantly different among all the gender groups. The estimated b of gross energy for all the gender groups was positive indicating that the fish were able to deposit energy even at the lowest feeding level (0.5%). The highest (best) estimated b was 31.2 g for the mixed-gender group, which indicated that the mixed-gender group extends the least energy. The b values for the crude fat and crude protein for the mixed-gender group were also the highest b values among the gender groups. The lowest predicted b value was for the female-gender group (b = 24.9 g), which was related to the low b value for both crude fat and crude protein, the most essential energy sources, compared to the other groups. Maintenance level predictions were not made for energy deposition for the gender groups. The predicted ML for all the groups was greater than 0 which means the actual ML was less than the lowest feeding rate (0.5%).

CONCLUSION

All-male Nile tilapia gender groups grow faster than female or mixed-gender groups. The differences in growth can be explained by the saturation kinetics curves generated during this study. Males exhibited higher R_{max} and $K_{0.5}$ for growth than female or mixed-gender groups because they used fat, protein and energy more efficiently.

Based on our results, methods to provide all-male tilapia for aquaculture should be used to maximize growth and performance.

The Saturation Kinetic Model is an easy tool to assess the nutritional requirement for fish and other animals around the world and especially in the developing countries.

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APPENDIX TABLES

Appendix Table 1. The dietary (intake) and fish (response) analysis for growth of *O. niloticus* for the three groups.

	Male		Female		Mixed gender	
Feeding Rate %	Intake (g)	Response (g)	Intake (g)	Response (g)	Intake (g)	Response (g)
0.05	27.8	3.5	23.8	6.5	28.5	1.0
0.05	25.6	3.0	22.6	8.0	27.9	2.0
0.05	25.3	2.0	22.3	9.0	29.4	0.0
1.0	55.4	12.0	43.5	10.0	40.5	13.0
1.0	57.0	13.0	58.3	5.0	62.9	32.0
1.0	53.4	16.0	54.0	15.0	59.2	34.0
2.0	117.9	82.0	106.3	45.0	73.9	59.5
2.0	129.4	74.0	104.2	58.5	125.2	131.0
2.0	144.9	108.0	99.1	58.0	131.7	93.5
4.0	237.2	146.0	266.6	99.5	244.4	140.0
4.0	248.4	182.5	290.6	105.0	294.0	126.5
4.0	299.0	136.5	269.6	107.5	319.2	125.5
6.0	530.5	188.5	491.0	169.0	461.6	169.0
6.0	518.7	209.5	476.3	172.5	405.7	170.0
6.0	499.1	217.0	475.0	187.0	429.7	178.5

Appendix table 2. The dietary (intake) and fish (response) analysis for protein of *O. niloticus* for the three groups.

Male			Female		Mixed gender	
Feeding Rate %	Intake (g)	Response (g)	Intake (g)	Response (g)	Intake (g)	Response (g)
0.05	12.9	-1.6	11.0	-1.9	13.2	2.2
0.05	11.9	-1.2	10.5	-0.5	12.9	4.4
0.05	11.7	-0.9	10.34	0.4	13.6	4.8
1.0	25.7	2.0	20.2	1.5	18.8	5.1
1.0	26.4	1.0	27.0	1.3	29.1	9.3
1.0	24.7	6.5	24.9	3.6	27.4	10.7
2.0	54.6	14.6	49.2	11.9	34.2	11.8
2.0	59.9	7.5	48.2	13.3	58.0	30.2
2.0	67.1	19.9	45.9	13.1	61.0	21.5
4.0	109.8	26.9	123.4	23.3	113.2	31.6
4.0	115.0	33.5	134.6	24.3	136.1	30.3
4.0	138.5	19.9	124.8	25.9	147.8	31.2
6.0	245.6	36.6	227.3	39.7	213.7	43.7
6.0	240.2	48.2	220.5	42.4	187.9	34.9
6.0	230.6	45.0	219.9	49.8	198.9	45.5

Appendix table 3. The dietary (intake) and fish (response) analysis for fat of *O. niloticus* for the three groups.

Male			Female		Mixed gender	
Feeding Rate %	Intake (g)	Response (g)	Intake (g)	Response (g)	Intake (g)	Response (g)
0.05	1.1	-1.7	0.9	-1.5	1.1	0.01
0.05	1.0	-1.3	0.9	-1.9	1.1	-0.1
0.05	1.0	0.3	0.9	-1.7	1.1	-0.1
1.0	2.1	0.8	1.7	0.5	1.6	1.4
1.0	2.2	1.4	2.3	0.8	2.4	6.3
1.0	2.1	2.0	2.1	1.0	2.3	5.8
2.0	4.6	7.7	4.1	6.2	2.9	6.2
2.0	5.0	5.8	4.0	4.0	4.8	15.7
2.0	5.6	10.5	3.8	6.0	5.1	14.2
4.0	9.2	13.6	10.3	11.1	9.4	17.6
4.0	9.6	15.9	11.2	12.9	11.4	28.6
4.0	11.5	10.5	10.4	13.5	12.3	18.2
6.0	20.5	26.0	19.0	20.8	17.8	40.8
6.0	20.0	30.8	18.4	20.8	15.7	41.8
6.0	19.2	33.5	18.3	21.9	16.6	38.7

Appendix table 4. The dietary (intake) and fish (response) analysis for ash of *O. niloticus* for the three groups.

Male			Female		Mixed gender	
Feeding Rate %	Intake (g)	Response (g)	Intake (g)	Response (g)	Intake (g)	Response (g)
0.05	2.4	-0.9	2.0	-1.4	2.4	-0.6
0.05	2.2	-0.8	1.9	-1.0	2.4	-0.7
0.05	2.2	-0.6	1.9	-0.9	2.5	-0.5
1.0	4.8	0.4	3.7	0.03	3.5	0.6
1.0	4.9	0.1	5.0	-0.5	5.4	2.6
1.0	4.6	1.4	4.6	0.4	5.1	2.6
2.0	10.1	7.1	9.1	3.1	6.3	5.6
2.0	11.1	3.8	8.9	5.4	10.7	9.6
2.0	12.4	7.6	8.5	6.4	11.3	15.0
4.0	20.3	12.4	22.8	12.7	21.0	13.6
4.0	21.3	17.1	24.9	12.0	25.2	14.4
4.0	25.6	11.2	23.1	13.4	27.4	15.7
6.0	45.5	24.2	42.1	24.6	39.6	25.3
6.0	44.5	37.9	40.8	28.9	34.8	23.4
6.0	42.7	20.4	40.7	33.6	36.8	24.0

Appendix table 5. The dietary (intake) and fish (response) analysis for gross energy of *O. niloticus* for the three groups.

	Male		Female		Mixed gender	
Feeding Rate %	Intake (Kcal)	Response (Kcal)	Intake (Kcal)	Response (Kcal)	Intake (Kcal)	Response (Kcal)
0.05	123,98	314,206	106,315	251,819	127,234	370,344
0.05	114,276	304,348	100,851	325,155	124,424	351,401
0.05	113,028	275,786	99,602	259,525	131,293	439,543
1.0	247,287	477,516	194,208	394,913	180,782	290,950
1.0	254,156	581,175	260,088	518,206	280,383	651,720
1.0	238,232	590,416	239,793	428,795	263,835	611,224
2.0	525,796	873,900	473,966	676,607	329,716	497,362
2.0	577,002	877,601	464,599	696,769	558,269	1,155,540
2.0	646,318	1,028,315	442,119	634,615	587,618	946,145
4.0	1,057,838	1,110,456	1,188,974	1,037,963	1,090,310	1,139,686
4.0	1,107,794	1,362,101	1,296,382	1,146,432	1,311,369	1,199,072
4.0	1,333,849	1,208,868	1,202,713	1,088,405	1,423,772	1,201,901
6.0	2,366,084	1,641,277	2,189,986	1,628,441	2,058,849	1,539,204
6.0	2,313,629	1,719,014	2,124,418	1,558,574	1,809,689	1,462,823
6.0	2,221,834	1,649,984	2,118,797	1,527,827	1,916,472	1,598,685