

Azolla A Boon for the Growth of Gift Tilapia, *Oreochromis Niloticus* (Linnaeus, 1758)

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Abstract

Understanding the nutritional requirements and production of fish feed is essential to the development and sustainability of aquaculture for the matured feed mill practices. Fish feed management includes choosing the right feed, using a correct feeding method, calculating the feeding cost and ensuring the cost effectiveness of fish farm. The purpose of the present study was to feed the fishes with four different feeds and assessment of growth performance by length weight relationships. The growth analysis was executed by measuring FCR and related indices in gift tilapia *oreochromis niloticus*. Four feed composition was selected without any chemical contamination. In the present study fishes were collected from the golden farm, Neyyattinkara, Thiruvananthapuram district, South India. Fishes were introduced into four different tanks of well oxygenated good quality water. In each experimental tank, 20 fishes were introduced. For each experimental container a specific feed was fixed from 4 different diets. Every day feed was given to the fishes. They were fed two times for the period of 3 months. They were maintained to assess their growth performance. Four types of feeds used for the present study are control diet, diet 1, diet 2 and diet 3. Measurement of length and weight was measured on every 15 days interval. Based on the length-weight data, growth analysis was carried out by calculating relative growth (RG), relative growth rate (RGR), specific growth rate (SGR), feed conversion ratio (FCR) and the Fulton's condition factor. The results of mean length in diet3 proved the highest length of $6.455 \pm 0.46\text{cm}$ and diet one contain lowest length of $5.03 \pm 0.68\text{cm}$. Highest weight of $96.8 \pm 0.37\text{g}$ was recorded in diet3 and lowest weight obtained for the control diet ($3.645 \pm 0.306\text{g}$). In growth analysis the relative growth of fish was estimated in diet 3 having high relative growth (11.91). Relative growth rate was elevated in diet 3 (1.5597). The upper limit of specific growth rate was 1.9146% observed in diet 3. The feed conversion ratio was maximum in diet 3 (58.06) and minimum in control diet (36). The highest k value in Fulton's condition factor was noticed in diet 3 (88.32). All experimental results proved that live Azolla [D3] was a superior feed for the present trials.

Introduction

The raise of human inhabitants has led to the scarcity of animal protein sources all over the globe. It has directed the awareness of fish as the fast and strong compensatory source of high quality animal protein. Fishes are quite diverse from the other animal food sources because they provide calories with high class proteins which hold all essential amino acids in simply digestible form. So they are the valuable nutrition source. Numerous studies have investigated the probability of optional protein source (Gatlin *et al.* 2007).

Health promoting aspects of fish in the human diet with Omega 3 fatty acids and other vital ingredients were contributed by aquaculture. Fish meal is a scarce and expensive protein source for commercial fish feeds. This explains the need for the substitute of fish proteins by other sources. Most of these studies showed that the partial replacement of fish meal can be successfully accomplished with respect to nutritional and health aspects.

Supplemental feeding is necessary for the enhancement of fisheries to achieve greatest yields from resources of fresh water fish farming. Replacement of improved nutrients can instigate fish growth rapidly and they accomplish maximum weight in shortest possible time. The nutritional content of the feed entail on what species of fish is being cultured and at what stage it is cultured. Fish nourishment has advanced in recent years with the development of latest, balanced, profitable diets that encourage optimal fish growth and health. When fishes are reared in high density in indoor systems or confined cages, they cannot scavenge freely on natural food.

Abwao *et al.* (2017) reported the effect of substituting hydrolyzed feather meal for fresh water shrimp meal on growth, apparent digestibility and body composition in tilapia *Oreochromis mossambicus*. The evaluated results recommended its 10% substitution. Jim *et al.* (2017) studied the comparative analysis of nutritional values of *O. niloticus*. The results suggested the effluent from sewage and fertilizer industries caused pollution and proliferation of water hyacinth contributed the pervasion of chemical composition of the fish. Velasquez *et al.* (2015) conducted a trial to evaluate the effects of Spirulina (*Arthrospira platensis*) inclusion in experimental diets of juvenile Nile Tilapia (*Oreochromis niloticus*). The results indicated that the optimal level of dietary replacement for increased growth performance improved feed utilization efficiency and enhanced overall health status of juveniles. Mustafa *et al.* (2016) reported the effect of dietary supplementation with Azomite, a natural mineral of volcanic ash on growth performance, innate immune response and disease resistance in *O. mossambicus* against *Aeromonas hydrophila*. Azomite projected the capability to support all functions. Onder *et al.* (2014) conducted a study to determine the effects of diets replacing fish meal (FM) with Peanut meal (PNM) on feed utilization, growth performance and body composition of Mozambique tilapia (*O. mossambicus*). Their results indicated the substitution of 20% dietary FM with PNM had no adverse effects on the growth, body composition parameters and general health of Mozambique tilapia fries. Oliver *et al.* (2008) conducted a study to concentrate on the use of the micro alga *Spirulina maxima* as a protein source in diets for tilapia, *O. mossambicus* fry and it was observed that Spirulina can replace up to 40% of the fish meal protein in tilapia diets.

Gangadhar *et al.* (2015) studied the effect of azolla incorporated diets on the growth and survival of *Labio fimbriatus* during fry to fingerling rearing. The study indicated that the possibility of incorporating azolla in diets of *L. fimbriatus* up to 40% resulting in savings of feed cost. The aquatic fern Azolla can grow without nitrogenous nutrients because of the presence of the N₂-fixing ability of symbiotic Anabaena. Djissou *et al.* (2017) studied the effect of total replacement of fishmeal by earthworms and *Azolla filiculoides* meals in the diets of Nile tilapia, *Oreochromis niloticus* which were reared in concrete tanks. In their experiments the mean weight were lesser than the control and best growth performance and feed utilization were obtained in fish fed test diets.

Felicitta *et al.* (2013) studied the influence of *Allium sativum* (Garlic) and *Allium cepa* (onion) on growth, survival and hematological parameters of *Oreochromis mossambicus*. The results suggested that 3% garlic in fish diet can improve growth and disease prevention in *O. mossambicus*. Jisr *et al.* (2018) reported the length weight relationship and relative condition factor of fish inhabiting the marine area of

the Eastern Mediterranean city, Lebanon. The results indicated that almost all the captured species exhibited a thinner growth and negative allometry. Leonard *et al.* (2012) studied the length weight relationships for 36 freshwater fish species from two tropical reservoirs. The results indicated that the family cichlidae with the highest number of eight species. Amonodin *et al.* (2018) reviewed the length weight relationship of freshwater fishes in Malaysia. Their results influenced the environmental changes related to fish ecological health.

Typically in the formulation of feed, selected parameters were greatly influenced by the raw material selection by the introduction of alternative nutrition sources. Dietary changes using alternative sources can be utilized in the culture system. Moreover 40 - 60 % of the total feed production cost, any negative effects on feed efficiency should be avoided. Finally the quality of the product including its highly beneficial properties for human health should not be compromised.

Due to the demand of fish meat, to overcome the nutrition deficit and to get a good quality fish meat without any chemical contamination; it is necessary to substitute a superior feeding exploration. In the present study, assessment of growth was substantiated by measuring length-weight relationships and by applying statistical factors due to the interpretation of relative growth, relative growth rate, specific growth rate, feed conversion ratio and Fulton's condition factor. Gift tilapia, *Oreochromis niloticus* fishes were fed by mentioned diets. The present study was framed to assess the growth of fishes in three selected feeds with control and comparing them by utilising natural feed ingredients.

Materials And Methods

COLLECTION OF SAMPLE

For the present study purpose gift tilapia, *Oreochromis niloticus* was collected from the Golden farm, Neyyatinkara, Thiruvananthapuram district of Kerala. The collected fishes were maintained and cultured by using selected feeds.

MAINTENANCE AND CULTURE OF FISHES

In the present study 1cm mean sized fishes were introduced into different cement tanks of well oxygenated good quality water. In each experimental container 20 fishes were introduced. Four different diets were given to the fishes in four different tanks. All the experimental trials were done in triplicates and their average was taken for data analysis. Every day, they were fed two times. For the period of three months they were maintained to analyse different assessments of their growth performance.

FEED INGREDIENTS

Control Diet	Rice, ground nut oil cake, turmeric, corn, Green gram.
Diet 1	Rice, dry shelled fish, wheat, fenugreek and corn.
Diet 2	Growfin (readymade feed obtained from farm)
Diet 3	Azolla

FEED FORMULATION

Different ingredients selected to formulate the feed were separately sun dried and crushed well using a mixer grinder to small particle size. A sieve was used to remove large particles or foreign materials which can damage the machinery. The ingredients were weighed and appropriate quantities were taken for feed preparation.

1. Control Diet [CD]

The control diet was prepared by adding different amount of ingredients such as 25gm rice flour, 25gm ground nut oil cake; 5gm turmeric powder, 20gm corn flour and 25gm green gram were taken. The modified method of Gull *et al.* (2005) was adapted. With the help of warm water the dry ingredients were mixed slowly. Good mixing can improve palatability. Water was added and mixed well to form a mash with a cake like consistency. As a general rule, the total moisture content of the mash should be in the range of 45 to 55% to produce good pellets. Pass the feed mash mixture through a pellet machine (mincer) with 1mm diameter. The mash was then steamed in a food steamer for about 10 to 15 minutes. Later it is cooled and kept in the refrigerator for 48 hours. This mash was then dried in the room temperature and cut the extrusion (which looks like noodles) into similar length to the closest pellet diameter. The moist pellets were then dried to moisture content of 10% or less simply by spreading the pellets under the sun rays.

2. Diet 1[D1]

This diet was prepared by taking 25gm rice flour, 25gm powdered shelled fish, 25gm wheat flour, 10gm fenugreek and 15gm corn flour and mixed thoroughly to make an emulsion with the help of warm water. Mix with dry ingredients slowly. To improve palatability the contents were mixed well. By the addition of needed water the mash with a cake like consistency was formed. The total moisture content of the mash should be kept in the range of 45 to 55% to produce good pellets. Pass the feed mash mixture through a pellet machine (mincer) having 1mm diameter. The mash is then steamed in a food steamer for about 10 to 15 minutes. Later it is cooled and kept in the refrigerator for 48 hours. This mash is then dried at the room temperature and cut the extrusion (which looks like noodles) into similar length to the closest pellet diameter. The moist pellets were then dried to reduce the moisture content of 10% or less simply by spreading the pellets under the sun rays.

3. Diet 2[D2]

Grow out (commercial) feed was bought as a readymade feed from the aquarium to feed the fishes in the third tank.

4. Diet 3[D3]

Azolla (natural feed) were given to the fishes in the fourth tank directly without any modification of the feed.

FEEDING THE EXPERIMENTAL FISHES

Correct delivery of food is important to reduce feed waste. Underfeeding can result in loss of production while overfeeding results in feed wastage and can lead to deterioration in water quality. A serious decline in water quality can result in loss of stock and the need for corrective measures. The fishes were fed twice a day preferably after sunrise (7 to 9 am) and before sunset (4 to 6 pm). 2gm of feed was provided to fishes in each container every time.

ESTIMATION OF LENGTH - WEIGHT RELATIONSHIP

The length and weight of the fishes (Jisr *et al.* 2018) from each container were measured at every 15 days to estimate the length – weight relationship. Fish was measured in terms of weight gain and increase in length. Total length (TL) was measured to the nearest 0.1mm using 30cm ruler as the distance from the tip of the anterior most part of the body to the caudal fin while weight measurements were taken in gm using 0.01g sensitive weighing balance at an interval of 15days.

GROWTH ANALYSIS

1. Relative growth (RG)

It is used for the comparison of the size of similar organisms. RG is calculated with the formula quoted here.

$$RG = \frac{(W_2 - W_1)}{W_1}$$

2. Relative growth rate (RGR)

RGR is the growth rate relative to the size of the population. It is calculated by the following formula.

$$RGR = \frac{(W_2 - W_1)}{W_1 (t_2 - t_1)}$$

3. Specific growth rate (SGR)

The specific growth rate is defined as the rate of increase of biomass of a cell population per unit of biomass concentration. Weight was recorded in grams. It is calculated by using the following formula (Steel *et al.* 1997).

$$\text{SGR} = \frac{\text{Log}[\text{Final weight}] - \text{Log}[\text{Initial weight}]}{\text{Culture days}} \times 100$$

Culture days

4. Feed conversion ratio (FCR)

The success of an aquaculture practice is dependent upon many factors relating to the field of biology, engineering and economics. The feed conversion ratio is a remarkable tool for understanding the acceptability of given feed (Inayat and Salim, 2005). FCR is calculated using the following equation.

$$\text{FCR} = \frac{F}{W_f - W_0}$$

Where F is the weight of food supplied to fish during the study period; W_f is the live weight of the fish at the end of the study period; W_0 is the live weight of the fish at the beginning of the study period

5. Length- weight relationship

The Length - weight (log transformed) relationships were determined by linear regression analysis and scatter diagrams of length- weight were plotted. The length – weight relationship of the experimented fish was worked out as per cube law given by Le Cren (1951).

$$W = aL^b$$

Where, W= Weight of fish (g), L is observed total length (cm), 'a' is the regression intercept and 'b' is the regression slope.

The logarithmic transformation of the above formula is

$$\text{Log } W = \text{log } a + b \text{ log } L$$

6. Fulton's condition factor (K)

Fulton's condition factor (K) was calculated according to Htun- Han (1978) equation as per formula given below:

$$W$$

$$K = \frac{\quad}{L} \times 100$$

Where W= weight of fish (g), L= length of fish (cm).

Results

ESTIMATION OF LENGTH AND WEIGHT RELATIONSHIP

In the present investigation, an average length and weight of freshwater fish gift tilapia '*Oreochromis niloticus*' was studied for a period of 90days. The length was measured in centimetres and weight was estimated in grams. The maximum length of *O.niloticus* was 7.1±0.7255 in diet 3. The diet 2 fed fishes showed the moderate length 6.455±0.4647. The fish fed with control diet and diet 1 showed the minimum length 5.175±0.512 and 5.03±0.6840. In this study the *O.niloticus* showed the maximum weight of 9.6±0.373 in the D3 feed. Diet 2 showed the moderate weight (5.2±0.324) .The fish fed with the control diet and diet1 were having minimum weight 3.645±0.306and 3.965±0.171 [Tables 1-2].

Table 1 Mean length of different diet trials in <i>O.niloticus</i>(cm)				
Days	CD	D1	D2	D3
0	0.96±0.2121	0.9±0.2578	0.915±0.2636	0.82 ± 0.2760
15	1.48±0.2627	1.34±0.2223	1.79±0.308	1.16 ± 0.1462
30	2.06±0.4722	1.8±0.4006	3.345±0.4455	1.6 ± 0.248
45	2.91±0.5617	2.055±0.3584	2.645±0.2992	2.35 ± 0.2824
60	3.21±0.4370	2.565±0.4706	4.015±0.1597	3.63 ± 0.341
75	3.815±0.2617	3.86±0.5277	5.605±0.4806	5.4 ± 0.418
90	5.175±0.5128	5.03±0.6840	6.455±0.4647	7.1 ± 0.7255

Table 2 Mean weight of different diet trials in <i>O.niloticus</i> (gm)				
Days	CD	D1	D2	D3
0	0.395±0.102	0.275±0.168	0.515±0.1443	0.8±0.387
15	0.615±0.364	1.155±0.235	0.86±0.162	2.04±0.273
30	0.87±0.52	1.67±0.288	1.43±0.305	2.37±0.515
45	1.11±0.301	2.54±0.376	2.06±0.256	3.32±0.223
60	1.59±0.381	2.895±0.338	2.81±0.3605	5.41±0.428
75	2.61±0.572	3.4±9.316	4.1±0.276	7.34±0.229
90	3.645±0.306	3.965±0.171	5.2±0.324	9.68±0.373

GROWTH ANALYSIS

Growth of fish depends on a wide range of positive or negative impact factors. Many studies are evidences for the growth of fishes in aquaculture. They mainly proved that increase in size of organisms directly proportional to feed consumption and quality of diets.

1. Relative growth

In the present study figure 1 showed the relative growth of fishes. The RG of the gift tilapia *O.niloticus* was high in the diet 3 (11.91) and less in the control diet (7.73).

2. Relative growth rate

The control diet having relative growth rate 0.1031 in CD and the diet1 has the RGR of 0.5385. The relative growth rate of 1.1182 was noticed in diet 2. The maximum RGR was 1.5587 in D3 [figure 2].

3. Specific growth rate

The specific growth rate of the *O.niloticus* was maximum in the diet 3 (1.9146) and minimum growth rate in control diet (0.8024). Figure 3 showed the results of SGR.

4. Feed conversion ratio>

The feed conversion ratio of the control diet was 36. Diet 3 have the feed conversion ratio of 58.06 and diet1 and diet2 ratio were 42.86 and 47.

5. Length weight relationship

The relationship between the length and weight of the control diet was $0.2931 \times L^{1.5235}$, diet1 was $0.002 \times L^{4.483}$. The diet 2 interpreted was $1.9503 \times L^{0.3608}$ and diet 3 showed the length weight relationship of $4.7025 \times L^{0.110}$.

6. Fulton's condition factor

The Fulton's condition factor for control diet 1 was 64.80 and diet 1 was 66.67. The diet 2 and diet 3 having the Fulton's condition factor of 72.96 and 88.32. This also indicates the changes in food reserves

Discussion

Unique set of water chemistry needs is indispensable to a healthy balanced and practical aquaculture system (DeLong et al. 2009). The growth of different fish species was ideal, which were influenced by diverse choice of water quality features (Nailor et al. 2021). Organisms generally augment in size (length, weight) during development. The key factors that influence the growth of fish are the amount of food, the number of fish utilizing same food, temperature, oxygen and other water quality factors. Besides these the size, age and sexual maturity of the fish also plays well. The rapport between the applied length and weight gained significance, because every animal gained growth by length and weight in its life. In the present investigation, an average weight of *O. niloticus* was studied for a period of 90 days. Maximum mean length 7.1 ± 0.725 cm was noticed in diet 3, minimum length found was 0.96 ± 0.212 cm in diet 1 and the moderate length was 5.03 ± 0.6840 cm in diet 2. Similar study carried out by Anene (2005) proved that the maximum weight of *Labeo rohito* observed was 1.964 ± 6.212 and minimum of 1.255 ± 3.971 in the carbohydrate diets. However the maximum length of *Labeo rohito* analyzed was 0.293 ± 0.928 and minimum of 0.172 ± 0.544 in the maltose dietary components. The Mean weight of *O. niloticus* showed the maximum of 9.68 ± 0.337 gm in diet 3 and minimum of 3.96 ± 0.168 gm in diet 2 and moderate (5.2 ± 0.324 gm) in diet 3. Compared with these studies, *O. niloticus* had the maximum length and weight in the present study. Ngugi et al. (2007) reason out the difference in length and weight gain of fish could be attributed to ideal conditions of the fish pond. The quality of water in the pond, feed availability and stocking density may have favoured development in the pond. In terms of cost efficiency low grade layers could be a good source of feed ingredient for commercially intensive tilapia, *O. niloticus* farming. Low labour value with no commercial worth poses serious environmental problems (Ishihara et al. 2008). Therefore unexploited D2 and D3 were used as a good fish diet source (Yangthong et al. 2014). Growth of fish depends on a wide range of positive or negative impact factor studies. Size increase of fish in aquaculture mainly depends on feed consumption and quality (slawaski et al. 2011). The relative growth of the gift tilapia *O. niloticus* was high in the diet 3 (11.91) and less in the control diet (7.73). The relative growth rate was higher in the diet 3 (1.558) and lesser in the control diet (0.1031). The specific growth rate of the *O. niloticus* was high in the diet 3 (1.9146) and minimum in control diet (0.8024). Similar study carried out by Makori et al. (2017) proved that the specific growth rate of the *O. niloticus* in earthen ponds were recorded the same growth rate of 3.7%/day SGR. When compared with these studies, our study reported moderate SGR. The length weight relationship of fish is important for estimation of growth observations. The relationship between length and weight of the control diet was $0.2931 \times L^{1.5235}$, the diet 1 having $1.9503 \times L^{4.483}$, the diet 2 having $4.7025 \times L^{0.110}$. Similar study was carried out by shahabuddin et al. (2015). They proved that the length and weight relationships of the juveniles of Nile tilapia, *Oreochromis niloticus* showed the allometric increase of growth. Khallaf (2003) reported differences in length weight relationship of *O. niloticus* in a polluted canal compared with those of other

authors in different localities and times. These differences were attributed to the effect of eutrophication and pollution on growth and other biological aspects. The Fulton's condition factor for *O. niloticus* was estimated in the range of 64.80, 66.67, 72.68 and 88.32 respectively. Further the highest amount was recorded in diet 3 (82.32) and the lowest amount was noticed in the control diet. The condition factor is an important factor to determine the relative degree of robustness and nourishment in fish (Mortuza and Al-Minsed, 2013). This factor might be influenced by sex, age, maturity and environmental condition (Anyanwu and Okoro, 2007). The condition factor obtained in the present experiment showed fish above average condition which indicated good health condition during the experiment and indicated an isometric growth, which is the desirable factor in fish farm. All the results of the present study proved that there was a significant increase in the body size of fishes reared in azolla [D3]. So from the present results exposed that *O. niloticus* will grow well in azolla fed tanks.

Declarations

Funding

No funding was received

Conflicts of interest/Competing interests

There are no conflicts of interest to declare.

Ethics approval

The Department of science, Southern Region has approved this research.

Contribution Description

LGB conceived and designed research. MR conducted experiments and generated data. LGB and MR analyzed data. MR wrote the manuscript and LGB corrected and edited the whole contents. All authors read and approved the manuscript.

Data Availability Statement

Data available as supplementary data

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Figures

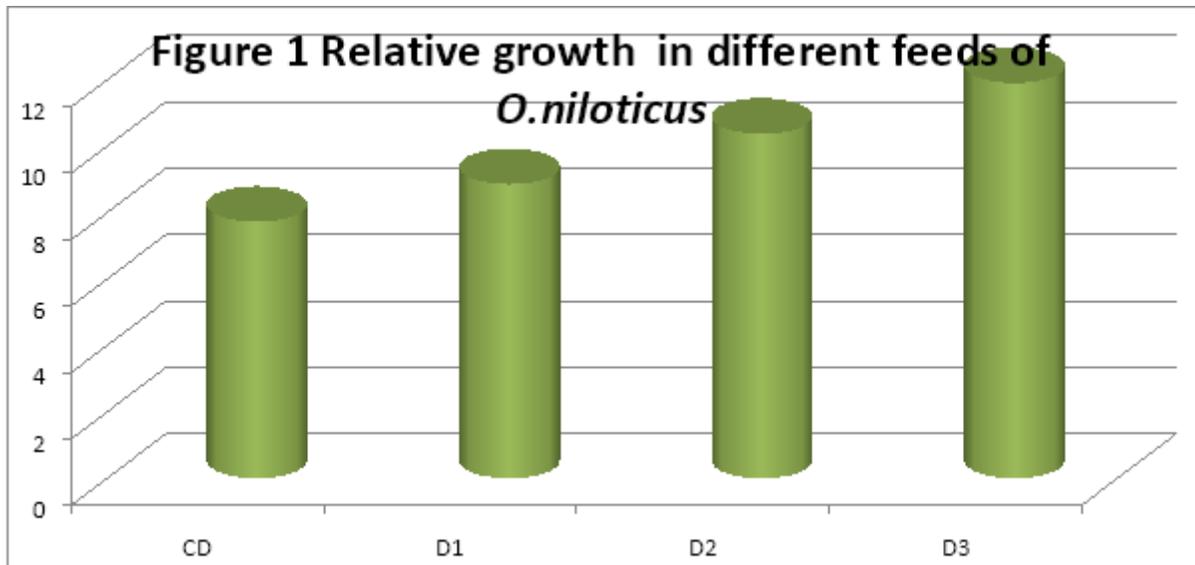


Figure 1

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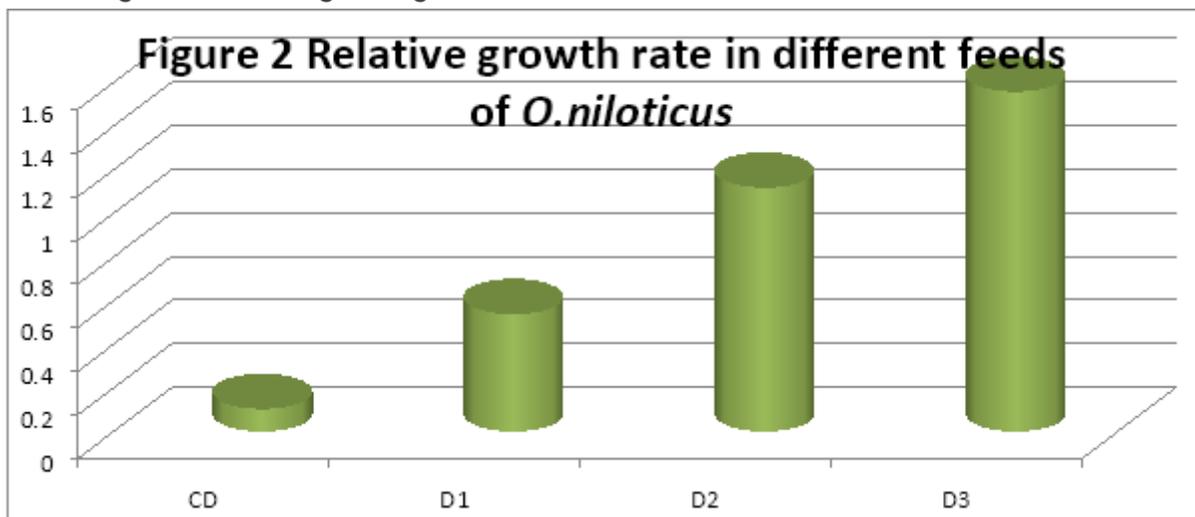


Figure 2

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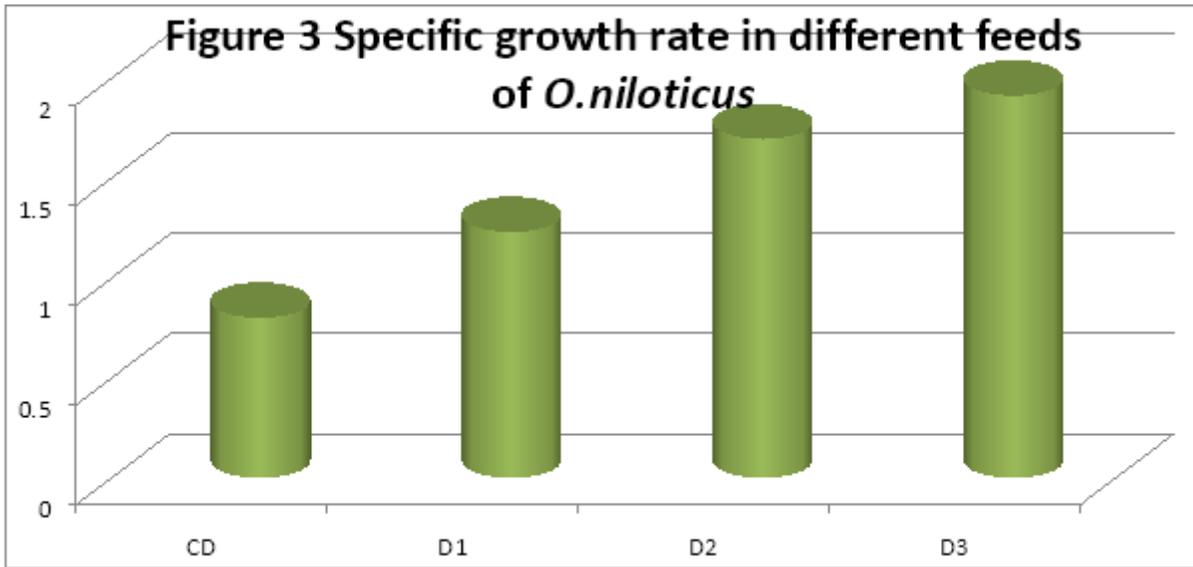


Figure 3

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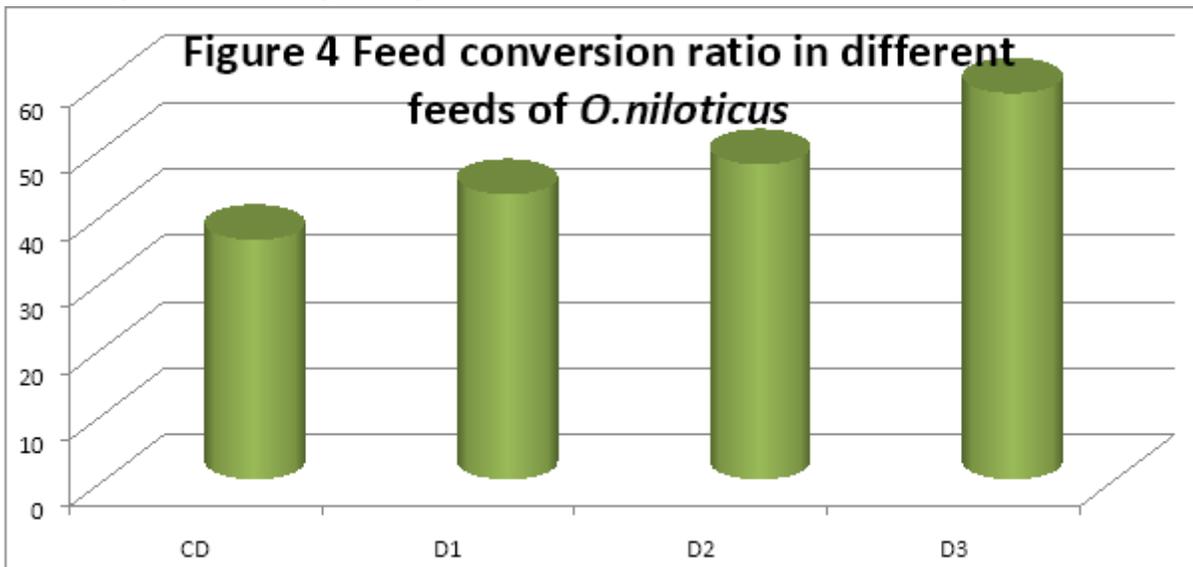


Figure 4

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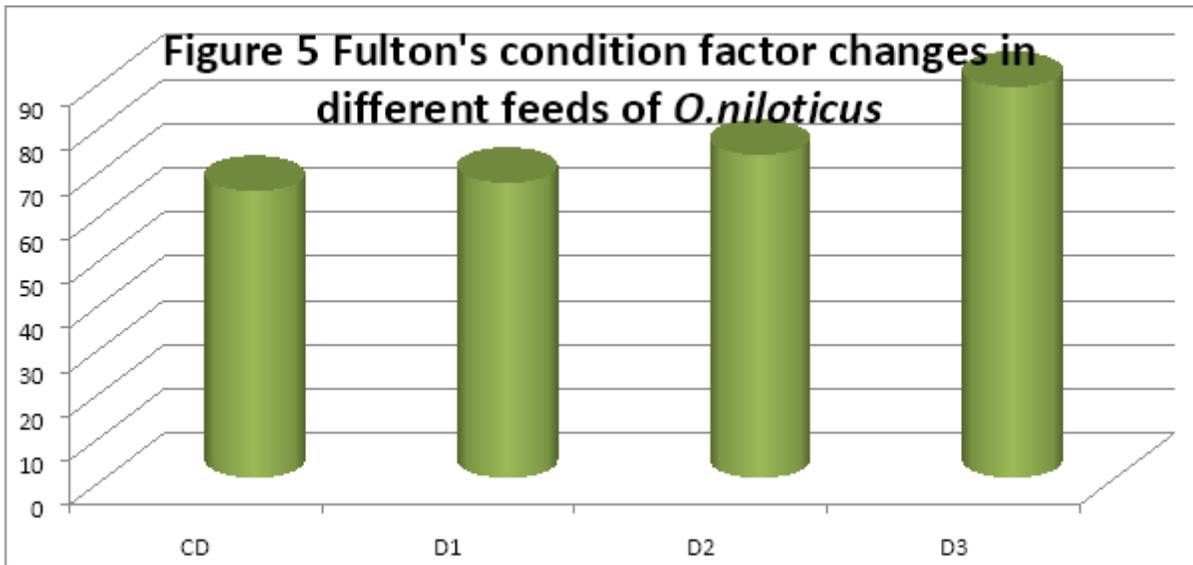


Figure 5

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