

Effect of protein levels in Tilapia (*O. niloticus*) diets on its growth performance in the aquaponics system in semi-greenhouse conditions

Mohammad N Wade¹ MA Salam^{2*} Mohammad M Haque³

1. *PhD student. Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, 2202. Bangladesh.*

2. *PhD Supervisor. Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh.*

3. *PhD Co-supervisor. Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, 2202. Bangladesh.*

4. **Corresponding Author: masalambau@gmail.com; salamaqua@bau.edu.bd*

Abstract

The cost of protein sources renders fish feed expensive, hence aquaculture. Tilapia(*Oreochromis niloticus*) was cultured in aquaponics (ability to withstand overcrowding) in order to explore potentials which may minimize the feed cost in aquaculture. A diet with 15, 25, 35 and 32% protein levels was formulated according to treatments T_1 , T_2 , T_3 and T_{con} to assess the effect of the protein levels on growth of the fish. Ingredients used were rice bran, maize meal, soya beans meal, fish meal, vitamin premix, methionine, lysine, vitamin C, vegetable oil and cassava flour (binder). Pearson square method was used in calculating the ingredient proportion. The feed conversion ratio in treatments $T_1 \& T_2$ was '1.2' and, it was '1.0' in treatments $T_3 \& T_4$. The specific growth rate was highest in treatment T_3 (4.63) followed by treatment T_1 (4.0) and T_{con} (4.18). The condition factor was highest in treatment T_1 (0.39), and lowest in treatment T_2 (0.34). The 'b' exponent value of the regression equation was highest in treatment T_1 (3.618), and lowest in treatment T_3 (2.6386). The study suggests that 15% protein level (treatment T_1), effects a more sustainable growth, having recorded the highest 'b' exponent value, highest 'K' value, tolerable FCR and SGR.

Keywords

Protein levels, aquaponics, tilapia, growth, ingredients, regression

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I. Introduction

As a result of the need to produce more and good quality food, without further damage to the natural environment, there has been an exploration of soilless agricultural systems, most popular, include aquaculture, hydroponics and most recently aquaponics system (Kratchy, 2009). Aquaponics is a system in which plants in a hydroponic system utilize organic fertilizer from aquatic animals such as fish, crustaceans, mollusks, amphibians, reptiles, etc., through the biological functions of symbiotic bacteria, thereby cleaning the water for the animals to use again in a circulation system. According to Mchunu, Lagerwall, Serzane, (2017) aquaponics opens a new niche for sustainable food production which is necessary for many countries in the world today.

Tilapia is reported as the second most important group of farmed fish after carps; but, it is the most widely grown of any farmed fish in the world (FAO, 2012). Its hardiness and adaptability to a wide range of culture systems led to commercialization of its production in more than 100 countries. Tilapia has assumed a princely position in the wake of aquaponics, due to its wide temperature tolerance range (Iwalewa, 2011) and its ability to withstand overcrowding (Rakocy, Masser, Losordo, 2006). As an omnivorous feeder (Fagbenro, Fasasi, Jegede, Olawusi-Peters, 2011) which accepts feeds made with agro-industrial by-products, tilapia produces huge wastes per unit of time rendering it an excellent candidate for aquaponics production.

Fish feed is usually seen as the most important expenditure item in aquaculture (FAO, 2012; Wade & Ibrahim, 2015); there is therefore the need to further explore all potentials to reduce its expense to the barest minimum. In a study by Rakocy, Bailey, Cole, Shultz (1997) the feed protein content of Tilapia was reported as 32%. Omnivorous fish such as tilapia and Carps need 25-35% protein in their diet while carnivorous fish need up to 45% protein in order to grow at optimum level was again reported in FAO (2014). Ariyaratne (2011) in his tilapia feed studies used 20% protein content. In view of the crucial status of proteins in fish feed formula and its concomitant cost compared with other feed ingredients, there is the need to try a lower protein percentage for

tilapia in aquaponics while envisaging economical production. This formed the bases for utilizing protein as a treatment factor in aquaponics experiment to assess tilapia (one of the two aquaponics products) growth with formulated feed having different protein levels.

II. Materials and Methods

2.1 Study Site

The study was conducted in the “Aquaponics Oases” Laboratory of the Department of Aquaculture in the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh; located at 24° 45' 0" North, 90° 24' 0" East, in Bangladesh. The Laboratory is a form of semi-greenhouse structure allowing access to direct sunlight for hydroponic beds at one side only. As this side was already occupied with one set of an experiment, the second side with available space but no direct sunlight was used for mounting the present experimental gadget.

2.2 Experimental setup

The set-up consisted of four treatments in triplicates, the forth was a control, making twelve units. Each unit consisted of a 300L plastic tank for fish rearing, a mini-submersible pump and two oxygen stones in it, the water level was however kept at 200L and a fish stock of 1.38 kg on the assumption that 50L of water is constantly pumped into the biofilter, mostly leaving the fish with just 150L. Mchunu (2017), earlier suggested 1.0 kg fish/100L of water (in this case the stocking density is kept constant throughout the experiment). The unit also consisted of a 60L plastic drum used as a biofilter; and a half-drum embedded with brick chips and sand used as a hydroponic (vegetable production unit) substrate (media). Although with media as a substrate, the use of a biofilter is unnecessary (Rokacyet *et al.* 2006) nevertheless, a biofilter was implored here for additional efficiency in nitrification. The substrate provided support for six Okra (*Abelmoschus esculentus*) seedlings transplanted in it and again acted as second home for bacteria. The feed components constituting the complete feed for tilapia were varied in protein content at three levels: 15, 25 and 35%, according to treatments, one, two and three, and were studied over a three-month period. The control treatment had 32% protein.

2.3 Formulated diets

The ingredients used were first submitted to the Nutrition Laboratory under the Department of Aquaculture, BAU for proximate composition analysis (table 1) according to the methods of Association of Official Analytical Chemists (AOAC, 1980, 95/2002). The Pearson square method was implored in calculating the desired proportions, where the contribution of every feed ingredient or feed supplement (trace elements) in the formulation of fifty kilograms (50kg) feed per treatment was clearly spelt. Ingredient quantities were then purchased based on the results of the calculations made. All ingredients were ground to required level and the formulation was done to the level of a marsh. The marsh was taken to Aquaculture System Laboratory of the Department of Aquaculture, BAU for pelleting to 2.0 mm and 4.0 mm sizes respectively. The pellets were then sun-dried for seven consecutive days and packaged in polyethylene-lined bags against moisture.

Table 1: Proximate Composition (Crude protein percentages).

Name of Ingredients	Sample number	Crude Protein % Calculation
Rice bran (R)	8	$3.8*0.2*0.014/0.5095*100*5.85 = 12.2$
	9	$3.6*0.2*0.014/0.5088*100*5.85 = 11.6$
Maize meal (M)	10	$2.6*0.2*0.014/0.5039*100*5.85 = 8.5$
	11	$2.5*0.2*0.014/0.5056*100*5.85 = 8.1$
Soya beans meal (S)	12	$11.4*0.2*0.014/0.5031*100*5.85 = 37.1$
	13	$11.6*0.2*0.014/0.5069*100*5.85 = 37.5$
Fish meal (F)	14	$12.0*0.2*0.014/0.5030*100*6.25 = 41.8$
	15	$12.0*0.2*0.014/0.5056*100*6.25 = 41.5$

As can be noticed in table 1 above, every ingredient has two samples, therefore the results with higher values were used for feed formulation calculation, which are as follows:

1. Rice bran (R) crude protein percentage = 12.2
2. Maize meal (M) crude protein percentage = 8.5
3. Soya beans meal (S) crude percentage = 37.5
4. Fish meal (F) crude protein percentage = 41.8 = 42.0

However, in feed formulation calculation using the Pearson square method, only the crude protein strength of feed ingredients is required, therefore, only the proximate composition of crude protein of ingredients was analyzed to its logical conclusion. Hence, the quantity of ingredients required to formulate fifty kilograms feed (also percentage equivalent) for each treatment is presented in table 2 below.

Table 2: Percentage composition of ingredient in treatment diets.

Name of Ingredients	T1(15% Protein)	T2 (25% Protein)	T3 (35% Protein)	TC (32% Protein)
Rice bran (kg)	23.5	13.5	4.5	7.5
Maize meal(kg)	16.0	10.0	3.5	5.0
Soya beans meal (kg)	3.5	11.0	18.5	16.5
Fish meal (kg)	4.0	12.5	20.5	18.0
Vitamin premix (g)	100	100	100	100
Methionine (g)	50	50	50	50
Lysine (g)	50	50	50	50
Vitamin C (g)	50	50	50	50
Vegetable oil (L)	0.25	0.25	0.25	0.25
Cassava flour-binder (kg)	2.5	2.5	2.5	2.5
Total	50	50	50	50

2.4 Fish feeding

Fish were fed at 3% body weight and the feed was administered at three ranges daily: 9.00-10.00am, 1.00-2.00pm and 5.00-6.00pm.

2.5 Water quality

Water quality in aquaponics is a sensitive issue because waste products are not directly discarded, instead, they are directed into a different compartment so as to be acted upon by denitrifying microbes and again directed into the hydroponic section where the usable products are absorbed, hence cleansing the water. For this reason, parameters like Dissolved Oxygen (DO), the measure for acidity and alkalinity termed the Power of Hydrogen atom (pH), Temperature, Ammonia (NH_3), Nitrites (NO_2) and Nitrates (NO_3)(table 3) were closely monitored. These parameters are critical in aquaponics, where, Steve(2006) and Lee Rinehart (2010) both observed that the stocking density of fish, feeding rate and growth rate can elicit rapid changes in water quality, therefore constant and vigilant monitoring is essential, to avoid stress. Data were weekly sampled throughout the study period and were analyzed.

2.6 Growth data

The fish total lengths were measured in centimeters using a 30cm ruler at the interval of two weeks and, the weights were measured using an electronic balance with a precision as low as 0.01g.

2.7 Growth indices calculated in the analysis

1. Specific Growth Rate (SGR) given by: $SGR = \ln W_2 - \ln W_1 \div \text{Culture days} \times 100$ (Brown, 1957).
2. Feed Conversion Ratio (FCR) given by: $FCR = \text{Amount of feed fed (kg)} \div \text{Weight gain (kg)}$ (Hepher, 1988).
3. Length-weight relationship given by: $W = aL^n$ Where W = weight, L = length, a = constant and n = exponent, which usually lies between 2.5 and 4.0 (The Cube Law, Le Cren, 1951). It is found (Le Cren, 1951) that for an ideal fish which maintains the same shape in the course of its growth, n=3, and this formed the bases on the assumption of isometric growth in fish ($H_0: b = 3$).

The Cube law formula was first transformed logarithmically (table 4)to allow expression of the lengths and weights graphically (Figure 1)and thus: If, $W = aL^n$, then, $\log W = \log a + n \log L$ (where n represents the slope of the line and log a, its position or the intercept).

The length-weight relationship was therefore determined using linear regression and a scatter plot fitted with a line of linearity. According to Le Cren (1951) the points for fish having the same length-weight relationship will lie on a straight line with some scatter due to individual variation. This line represents the logarithmic form of the Cube law equation ($W=aL^n$) as indicated above, where changes in the value of 'n' can usually be observed as changes in slope.

4. The Condition factor (K) given by: $K = W \div L^3 \times 100$, where W= weight of fish (g), L= length of fish (cm). (Froese, 2006).

2.8 Data Analysis

The length and weight of the specimens were first submitted to explorative data analysis. This included ANOVA where the difference in the mean sizes of fish in all treatments was tested (table 5). It was then followed by Post Hoc test to portray the significant deviations from expectation ($\alpha=.05$) in the distribution of lengths and weights of fish in the treatments (table 6a & 6b). The test for homogeneity of variances was done to reveal any significance in the deviation of lengths and weights from a normal distribution (table 7). Then the Kolmogorov-Smirnovtestwas implored for testing normality in the distribution of lengths and weights across the treatments (Table 8). Using linear regression (Ricker, 1973), the parameters for log transformed equation were

estimated. The statistical significance of the slope 'n' also represented as (b), the intercept (a), and the correlation coefficient (r) were analyzed using student's *t*-test (Rodriguez, 2017) in order to establish the level of confidence. The *t*-test was implored to check whether the fish differed from the isometric growth (H_0 : b=3). The comparison between the obtained values of *t*-test and the respective critical values (Datta, 2013) allow the determination of the 'b' value's statistical significance, and their inclusion in the isometric rating (b=3), positively allopatric (b>3) or negatively allopatric (b<3). IBM SPSS Statistics Version 20 and Excel windows 10 software were used for the analysis.

III. Results

Generally, dissolved oxygen level across the treatments was maintained at almost 6.0 mg/L on the average by means of aeration machines fixed for the purpose. The pH and Temperature also hovered around acceptable ranges (table 3) in all the treatments as the experiment was conducted in the summer. Differences between the treatments were insignificant ($P>0.05$). Parameters like ammonia and nitrites really had high figures on the average, but the effect of such elevated values were momently in a recirculating system like aquaponics and therefore elicited no negative consequences as indicated by the fish feeding actively. Nitrate values on the other hand were indicative of the unused fertilizer by the plants at any moment. As indicated, no variance within the groups in all treatments and the differences between them were insignificant.

Table 3: Water quality parameters of treatments^{a,b}

Parameter	T1 (15% Protein)	T2 (25% Protein)	T3 (35% Protein)	TC (32% Protein)
Dissolved Oxygen (mg/l)	5.95 ± 0.32	6.00 ± 0.00	6.00 ± 0.00	5.95 ± 0.32
pH	7.64 ± 0.40	7.64 ± 0.40	7.64 ± 0.40	7.64 ± 0.40
Temperature (°C)	29.3 ± 1.3	29.5 ± 1.2	29.6 ± 1.3	29.5 ± 1.3
Ammonia (mg/l)	0.91 ± 1.14	0.81 ± 1.05	0.60 ± 0.68	0.92 ± 1.18
Nitrites (mg/l)	0.99 ± 1.38	1.44 ± 1.98	1.11 ± 1.74	2.75 ± 2.40
Nitrates (mg/l)	22.85 ± 21.18	22.38 ± 21.56	30 ± 20.62	25.95 ± 17.92

a. No variance within groups

b. No significant differences between groups

Note: Tabulated values indicate Mean ± Standard deviation

As contained in table 4, the feed conversion ratio (FCR) values were observed to be '1.2' in T_1 and T_2 , and '1.0' in T_3 and the T_{con} . The specific growth rate (SGR) appeared highest in T_3 (4.63) which surpassed the T_{con} (4.18). Treatment T_2 showed the lowest growth rate (3.73), while T_1 had '4.0'. The length-weight relationship represented by the logarithmic equation, the constant or the intercept 'a', the slope 'b' of the regression, the correlation coefficient 'r' and the coefficient of determination ' r^2 ' were also presented in table 4, where the intercept of the regression line was observed to have altered a little from -1.3025 to -2.3902. The slope 'b' on the other hand varied between 2.6386 to 3.6108. The result of regressed lengths and weights of tilapia revealed the 'b' value higher than the critical isometric value '3' in T_1 , T_2 and the T_{con} . These were therefore commensurate with the positive allometric growth where fishes tend to be heavier but shorter in length. In T_3 however, the 'b' value appeared significantly lower than the critical value '3' indicating a form of slender growth of cultured fish (negatively allometric). At least 91% variability ($r^2 = 0.91$) in the growth model of tilapia in T_2 to 96% ($r^2 = 0.9681$) in T_1 compared with 98% variability ($r^2 = 0.9884$) in the T_{con} were explained by the coefficient of determination (r^2), the values of which (Datta, 2013) explains the goodness of fit of a Model. In all the treatments, over 95% proportion of variance in the dependent variable (weight) as shown by the correlation coefficient (r) is explained by the predictor variable (length). The logarithmic curves depicting the relationship between length and weight of tilapia in various treatments fitted with regression lines were presented in Figure 1. The linearity in the trends indicates same relationship between the lengths and weights of tilapia as earlier related.

In this study, the highest condition factor value observed was 0.39 in T_1 and lowest in T_2 (0.34). Treatment T_3 and the control T_{con} treatment each had 0.37 respectively. There were no significant differences between the treatments indicating some similarity in living conditions in all the aquaponic tanks.

On the analysis of variance (ANOVA), the result in table 5 indicated significance in the differences of sample means and numbers as well as variation within and between treatments while considering statistical standards. The Post-Hoc test at $\alpha=0.05$, (tables 6a & 6b) showed that the deviations from expectation in the distribution of lengths and weights of fish in all treatments were not significant. On the test for homogeneity of variances, the Levene statistics (table 7) indicated high significance in the deviations of lengths and weights from a normal distribution. The Kolmogorov-Smirnov test (table 8) on the other hand showed some significance in the normality of distribution in both lengths and weights in T_1 , T_3 and the T_{con} , but insignificant for T_2 .

Table 4: Length-weight relationship indices of tilapia in aquaponics

Treatments	Feed conversion ratio (FCR)	Specific Growth Rate (SGR)	Condition factor (K)	Logarithmic equation: LogW=loga+b log L	Correlation coefficient (r)	Coefficient of determination (r^2)	Slope (b)
Treatment one	1.2	4.00	0.39	Log W= log -2.3902 +3.6108 log L	0.984	0.9681	3.6108
Treatment two	1.2	3.93	0.34	Log W= log -1.9908 +3.2422 log L	0.954	0.91	3.2422
Treatment three	1.0	4.63	0.37	Log W = log -1.3025 +2.6386 log L	0.962	0.9263	2.6386
Control treatment	1.0	4.18	0.37	Log W = log -2.0099 +3.2469 log L	0.994	0.9884	3.2469

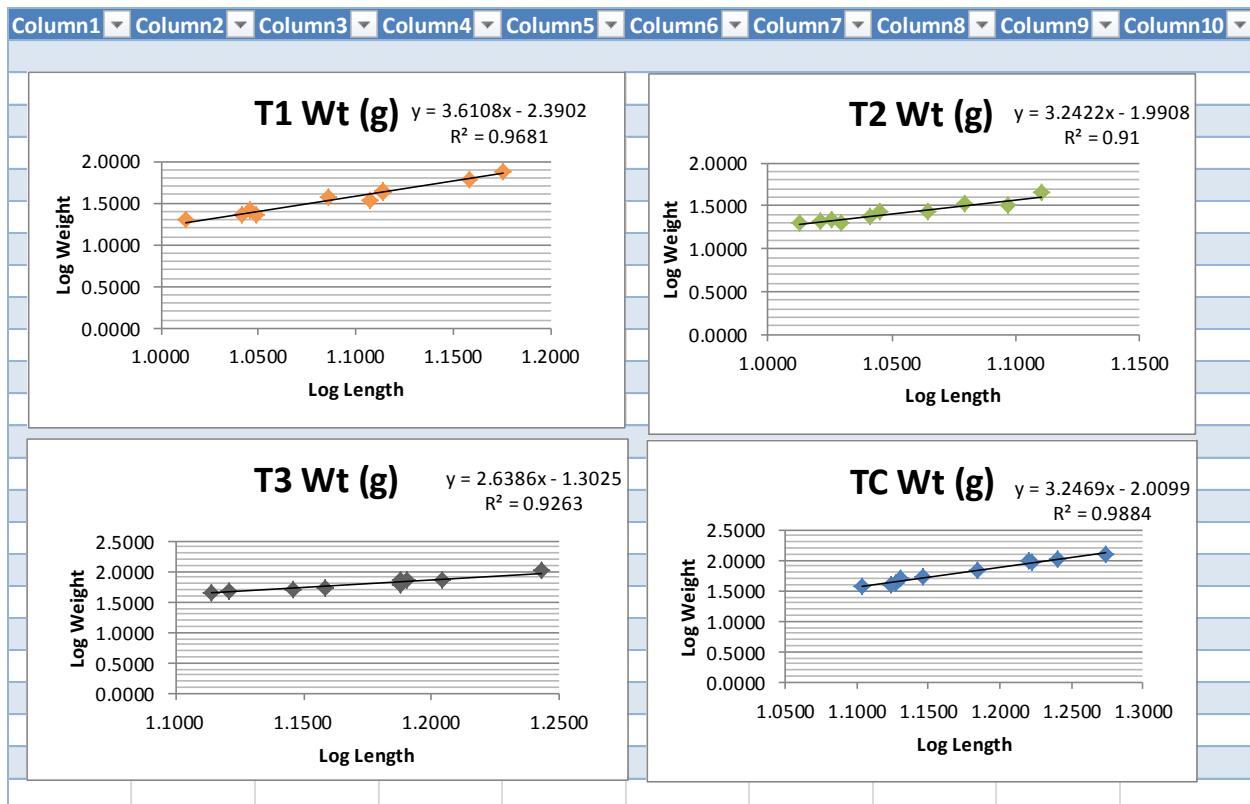


FIGURE1: Final logarithmic relationship between length and weight with regression equation of *Oreochromisniloticus* in aquaponics.

Table 5: ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Weight of the fish	Between Groups	34913.977	3	11637.992	31.825
	Within Groups	305713.933	836	365.687	
	Total	340627.910	839		
Length of the fish	Between Groups	377.582	3	125.861	29.867
	Within Groups	3522.874	836	4.214	
	Total	3900.456	839		

Post Hoc Tests

Homogeneous Subsets

Table 6a: Weight of the fish

Duncan		N	Subset for alpha = 0.05	
			1	2
Treatments				
1=treatment 1		210	23.9710	
2=treatment 2		210	25.1744	

4=CONTROL TREATMENT	210		35.8129
3=treatment 3	210		38.7313
Sig.		0.519	0.118

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 210.000.

Table 6b: Length of the fish

Treatment	N	Subset for alpha = 0.05		
		1	2	3
1=treatment 1	210	10.633		
2=treatment 2	210		11.168	
4=CONTROL TREATMENT	210			11.968
3=treatment 3	210	1.000	1.000	12.349
Sig.				0.058

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 210.000.

Table 7: Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Weight of the fish	35.309	3	836	0.000
Length of the fish	13.513	3	836	0.000

Table 8: Tests of Normality

	Treatment	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Length of the fish	Treatment 1	0.084	210	0.001	0.970	210	0.000
	Treatment 2	0.084	210	0.001	0.988	210	0.087
	Treatment 3	0.073	210	0.009	0.972	210	0.000
	Control treatment	0.118	210	0.000	0.972	210	0.000
Weight of the fish	Treatment 1	0.133	210	0.000	0.851	210	0.000
	Treatment 2	0.119	210	0.000	0.922	210	0.000
	Treatment 3	0.124	210	0.000	0.892	210	0.000
	Control treatment	0.172	210	0.000	0.849	210	0.000

a. Lilliefors Significance Correction

IV. Discussion

As reported by Rakocy (2006), 6.0-7.0 mg/L of DO levels achieve near 80% saturation in aquaponics culture tanks (Table 3). All compartments, from the fish tanks, biofilters harboring bacteria and the hydroponic beds for vegetables requires continuous supply of the gas. However, electric-power failure did halt aeration machines, sometimes in excess of one hour during the experimental period and therefore elicited fatal consequences particularly on the part of tilapia which greatly affected the survival rate. A similar report on pH and temperature in aquaponic tanks by Rakocy (2006) supports the present results. In a report by Endut (2011) nitrites tend to accumulate in recirculation water as a result of incomplete bacterial oxidation. Based on the capacity of the available motor pumping machines (2000L/hr.), the entire tank volume of water (200L) is pumped out in less than 15 minutes and continuously replaced by cleansed water from the hydroponic component of the system, hence lowering the effect of a possible stress on fish.

Perhaps, results from a number of studies (Hasan& Soto, 2017; Tacon and Metian, 2008; Jillian, 2018) also reported the FCR value of '1.0' and over; however, according to a report (FAO, 2006) tilapia should typically have an FCR of less than one, which is the bases of economic production, since fish diets are the biggest cost factor in intensive aquaculture. The FCR is noted (<https://itspro-bd.Blogspot.com/2015/07/definition-of-FCR>) to be a prime indicator of (a) Performance of the feed used, (b) Performance of the worker feeding the fish, (c) Performance of fish's health and (d) Cost effectiveness of using an existing feed.

Growth is said to be isometric ($b=3$) when the increase in weight of fish is proportionate with the increase in length (Froese, 2006). It is positively allometric ($b>3$), when the weight increases more than length (Wooten, 1992) and negatively allometric ($b<3$), when the fish length increases more than the weight(Pauly, 1984). As seen from the above results, T_1 and T_2 fed with 15 and 25% protein rich feed still tallied with the

T_{con} (32%) in achieving a similar growth pattern (heavier but shorter) unlike T_3 which was fed with 35% protein level feed, but grew slender. There could be something more than the protein levels which promotes isometric growth in particular if the present results are reliable. Perhaps, Ariyaratne (2011), in his feasibility studies on tilapia (GIFTstrain) in Sri Lanka reported that, in developing countries where labor costs are comparatively low, a significant saving in feed costs can be made by feeding diets with a lower protein content than that which is thought to be the optimal dietary protein requirement, without significant loss in growth or yield. However, the entire study can be said to agree with the findings of Le Cren (1951) and Froese (2006). The fact that the experimental data was from a fish culture source abates the cautionary note by Le Cren (1951) of not subjecting the length-weight data to any selection for weight against length. For instance, it happens in capture fisheries where gillnets may select the fatter among short fish and the thinner among long fish because this lowers the value of 'b' even though the means of length and weight may be unaffected. Slope variation may also be attributed to sample size variation or growth stage or even environmental conditions. The relationship between length and weight was again depicted by logarithmic curves as shown in Figure 1. The logarithmic trend lines at least lie on fairly straight lines (showing some linearity) with less scatter for all the treatments (Le Cren, 1951), indicating fish having same length-weight relationship. Such little scatter could probably be due to individual variation, and the differences in the value of 'b' due to changes in slope.

The condition factor was calculated as the ratio between the observed weight and that expected from the observed length. The bases of the expected weight were that for an ideal fish in whose length-weight relationship formula is $W = a L^3$, $n = 3$ obeys the cube law (Froese, 2006, Le Cren, 1951). Differences in condition factor have been interpreted as measuring various biological features such as fatness, suitability of environment or gonad development. Information on condition factor (Datta, 2013), can hence be vital to culture systems management because they provide the manager an insight on the specific condition under which fish is developing. However, comparing results of similar studies of tilapia in aquaculture ponds, values of condition factor in the present study were seemingly low. For instance, Laghari, (2011) obtained values up to '1.0' in tilapia study in concrete ponds. Datta, (2013) also reported condition factor values above '1.0' in a study of a different species (*Channapunctata*). Just as well, Rodriguez, (2017) recorded condition factor values below and above '1.0' in his Uruguayan coastal habitat study where he credited the condition factor as an indicator for habitat quality. Earlier however, (Laghari, 2011) reported a study on the reduction in the breeding and nursery grounds of *O. niloticus* in lake Turkana (Kenya), where it was observed that stress contributed to dramatically lower condition factor values. Still contained in Laghari (2011), an observation was made that pollution affects the condition factor of *O. niloticus* in a study in lake Mariut, Egypt. It could comparatively be understood that both stress and pollution are contentious issues in aquaponics as already indicated under water quality. These could have probably contributed in lowering the condition factors in all treatments in the present study.

V. Conclusion

In conclusion, the findings of this study indicated the growth performance of *O. niloticus* linked to other factors than increased protein levels in aquaponics. The least protein level (15%) treatment had an acceptable feed conversion rate, highest condition factor, highest 'b' value and optimum specific growth rate, as compared to the highest protein level (35%) treatment which perhaps had but highest specific growth rate. Notably, less protein inclusion in the former would mean cheaper production, hence sustainable. As a recommendation, it is worthwhile to make further findings on reduced stress and pollution in tilapia aquaponics.

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