academicJournals

Vol. 7(11), pp. 178-184, December 2015
DOI: 10.5897/IJFA15.0505
Article Number: 084FAF156900
ISSN 2006-9839

http://www.academicjournals.org/IJFA

International Journal of Fisheries and Aquaculture

Full Length Research Paper

Effect of dietary protein levels on ammonia concentration and growth of *Tilapia rendalli* (Boulenger, 1896), raised in concrete tanks

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Received 18 July, 2015; Accepted 9 December, 2015

***Tilapia rendalli* juveniles (±9.5 g) were cultured in concrete tanks to determine the effect of four dietary protein levels (30, 35, 40 and 45% crude protein (CP) in feed on ammonia concentration and growth performance of the fish, stocked at 15 fish per tank. Fish were monitored for a period of 90 days. Fish that were fed on 40% CP diet had significantly (P<0.05) higher weight gain, growth, and feed conversion ratio (FCR). Ammonia concentration was significantly higher (P<0.05) in tanks where the 45% CP diet was administered, and where also poor growth and survival rate was observed. Poor growth performance of the fish at inadequate (below 30% CP) and oversupply (above 40% CP) protein diet is evidence of the importance of taking precautions on the levels of protein inclusion in diet in tank culture. Higher dietary protein diet precipitates ammonia accumulation, thus compromising growth performance. Based on findings in this study, optimal protein level of 40% CP is recommended for tank culture.**

Key words: Ammonia concentration, dietary protein, growth performance, *Tilapia rendalli*.

INTRODUCTION

Tilapia rendalli (Red breasted tilapia) (Boulenger, 1896) is an important commercial species in sub Sahara Africa (Mair, 2001). It is the most commonly cultured species in Malawi alongside *Oreochromis shiranus*, *Oreochromis mossambicus* and *Oreochromis karongae.* The species is mostly preferred by farmers due to its flavour. *T. rendalli* is also the best candidate because it feeds on higher plants and has a reasonable growth rate when reared in extensive systems and supplemented with plant material compared to other species (Chikafumbwa, 1996). Chandrasoma and De Silva (1981) further observed that the length and weight at first maturity of *T. rendalli* varied from 18.8 to 25.8 cm and 126 to 380 g, respectively. Nyirenda et al. (2000) also observed that due to better growth performance, ability to breed, resistance to stress and marketing value, *T. rendalli* is ranked as the most potential cultured species for both commercial and small scale farming in Malawi. However, despite all advantages of *T. rendalli* over other species, little is known about *T. rendalli* nutritional requirement.

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Growth of fish in aquaculture systems depends on several factors, such as nutrition and the condition of water. Protein is the most essential nutrient component for growth performance, survival and yield of fish (Makwinja et al., 2013). On dry weight basis, protein makes up the maximum weight in fish body structure (Craig and Helfrich, 2009). Fish require protein for normal tissue function, maintenance and renewal of body protein and growth. However, because protein sources are costly, the fish need to utilise all protein provided in the diet for growth and muscle development. Furthermore, it is cost effective when protein is largely used for tissue repair and growth and less catabolised for energy (Gauquelina et al., 2007). Inadequate dietary protein in the diet reduces growth performance due to withdrawal of protein from less important tissue to maintain the functions of more important tissues (Halver, 1989). On the other hand, too much protein (above 40%) in the diet, results in only part being used to synthesise new proteins, while the rest is catabolised for energy (Alatise et al., 2006). Additionally, protein above 40% increases ammonia concentration in the culture systems.

Ammonia (NH_3) is a principle nitrogen excretory product of freshwater fish (Emerson et al., 1975). Its concentration in rearing tanks is due to end product of biological processes related with fish metabolism and is excreted mostly through the gills (Amoah, 2012). Large portion (over 80%) of the ammonia concentration in culture systems can also arise from the biological degradation of protein in waste feed and faeces by several genera of bacteria, including *Nitrosospira* and *Nitrosamines*. Other groups of bacteria, including *Nitrospira* and *Nitrobacter*, convert nitrite to nitrate (NO_3^-) (Tovar et al., 2000). Ammonia and its by-products are a major concern to fish health. Ammonia concentration above 0.05 mg/L may ultimately pollute the environment (Jindal et al., 2010) leading to multitude of symptoms and health problems in fish (Johnston, 2002). For instance, high concentration of ammonia (NH_3) (above 0.05 mg/L) in the culture water affects the diffusion gradient, which causes subsequent increase of ammonia concentration in the blood of the fish (Atle et al., 2003). Amoah (2012) observed that acute toxicity level of ammonia concentration (above 0.05 mg/L) in the tank systems can lead to hyper-ventilation, irregular swimming, convulsions and even death of the fish. This study was therefore designed to determine the effect of different protein levels in feed on ammonia concentration and growth of *T. rendalli* in concrete tanks.

MATERIALS AND METHODS

The study was conducted at the National Aquaculture Centre, Domasi, Southern Malawi (15°17'0" South and 35°24'0" East). The experiment was carried out in 1.5 m^3 concrete tanks laid out in a completely randomised design (CRD) with four dietary treatments (30, 35, 40, and 45% crude protein (CP)) replicated three times. The four dietary treatments were formulated using WinFeed version 2.8 (Mirza, 2004) by Linear Programming.

The ingredients (soybean, fish meal and maize bran) used in diet formulation were locally purchased from a local market in the area. Prior to formulation, the tested diets from each treatment were chemically analysed according to the standard methods of A.O.A.C (2003) for protein, fat, fibre and ash. In order to prepare diets, all the dietary ingredients formulations were finely ground and sieved using a 0.5 mm mesh size. Vitamin and mineral premixes, lysine and binders were included in the formulated diets at 0.2%.

To avoid contamination from previous feeds, the first feed from the metal die after a turn was discarded. The pellets obtained were then spread on a mat and sun-dried to constant weight for two to three days. The pellets were later broken down manually and sieved to bite size (0.1 and 0.2 mm) for fingerlings. All diets were labelled appropriately and packed in air-tight polyethylene bag and stored at a room temperature (25°C) for subsequent use.

A mixed sex *T. rendalli* juveniles of average body weight 9.8 g, were collected from National Aquaculture Centre Government Hatchery in Domasi, Zomba, Southern Malawi, during the month of October, 2014. The fingerlings were given a prophylactic treatment by immersing in salt (3% NaCl) solution for 10 min, to prevent spread and outbreak of viral, bacterial, fungal, and parasitic infections prior to introduction to growing tanks. During treatment, sufficient oxygen supply was maintained by changing and flushing out water frequently in the tanks. Before starting the experiment, the fish were acclimatised to the experimental condition for one week and fed on a commercial feed containing 25% CP.

T. rendalli juveniles were then stocked into 12 experimental tanks at 15 fish per 1.5 m^3 tank. Experimental diets (30, 35, 40 and 45% CP) were provided twice daily (10:00 and 15:00 h) for 90 days at 5% of fish live body weight. A record of supplied feed was kept for determination of feed conversion ratio (FCR) and protein efficiency ratio (PER).

Water quality parameters and ammonia

Water quality parameters in the tanks: temperature (Celsius thermometer), dissolved oxygen (Inolab Oxi Level 2 Oxygen metre), and pH (Suntex Model SP-701 pH metre), were monitored on daily basis at 8 am and 2 pm.

Ammonia was determined according to the EPA 350.1 and APHA 450-NH_3D methods (American Public Health Association, 2005). 500 ml of the reagent water was added to an 800 ml Kjeldahl flask. To reduce hydrolysis of cyanates and organic nitrogen compounds, 1 N NaOH was added to the 400 ml until the water sample was buffered at a pH of 9.5. 300 ml from the sample was distilled at the rate of 6 to 10 ml/min to form a solution of boric acid. Alkaline phenol and hypochlorite was added and reacted with ammonia to form indophenol blue that was proportional to the ammonia concentration. The blue colour formed was intensified with sodium nitroprusside and measured calorimetrically.

Fish sampling

Sampling was done every three weeks by catching the fish using a fine mesh scoop net, while removing excess water by gently blotting on a soft tissue paper. Fish were anaesthetized in crude clove powder (5 g/L) to reduce stress before weighing on Denver Instrument XL-3100 Scientific Balance Laboratory Scale. A sample of 30 fish was obtained from each treatment (10 fish from each replicate). Mortality of fish during the study period was recorded. After 90 days, fish were harvested and growth parameters were determined.

Growth evaluation

Weight gain, feed conversion ratio, specific growth rate, survival rate, protein index and condition factor were calculated according to Wang et al. (2005) as follows:

Weight gain (WG):

$$WG = W_f - W_{in} \dots\dots\dots\dots \quad (1)$$

where WG = weight gain (g), W_f = final weight (g), W_{in} = initial weight (g)

Feed conversion ratio:

$$FCR = \frac{Total\ feed\ consumed\ by\ the\ fish\ (g)}{Weight\ gain\ by\ fish(g)} \dots\dots \quad (2)$$

Specific growth rate (SGR (% day^{-1})):

$$SGR = 100 * \frac{log_e W_f - log_e w_{in}}{period\ of\ culture\ (day)} \dots\dots \quad (3)$$

Where W_f = final weight (g), W_{in} = initial weight (g)
Survival rate:

$$Survival\ rate = 100 * {}^{N_f}/_{N_{in}} \dots\dots \quad (4)$$

where N_{in} = Number of fish at the start of the experiment, N_f = Number of fish at the end of experiment.

Nitrogen metabolism (NM) was calculated as (Jamabo and Alfred-Ockiya, 2008):

$$NM = [0.54](b - a)h]/2 \dots\dots \quad (5)$$

where a = initial weight (g), b = final weight (g), h = experimental duration (days).

Protein Index:

$$PI = [(survival\ rate * (BW_f(g) - BW_{in}(g))/_T \dots\dots \quad (6)$$

where PI = Protein index, BW_f = final body weight (g), BW_{in} = initial mean body weight (g), T = experimental duration (days).

Protein efficiency ratio:

$$PER = \frac{Wg\ (g)}{wp\ (g)} \dots\dots \quad (7)$$

where W_g = weight gain (g), W_{p} = weight of protein fed (g).

Condition factor (CF):

$$CF = \frac{W_2}{L_2^3} * 100 \dots\dots \quad (8)$$

where W_2 = final fish average weight (g), L_2 = average total length (cm^3)

Regression model

Third order polynomial regression model was used to describe the relationship between the growth parameters and the dietary protein levels. Similarly, the relationship between ammonia concentration and the dietary protein levels was described using third order polynomial regression model with the following empirical model of third order:

$$Y_i = \beta_0 + \beta_{1_{x_1}} + \beta_{1_{x^2}{}_2} + \beta_{1_{x^3}{}_3} + \epsilon_i \dots\dots \quad (9)$$

where Y= dependent variable, β_0 is the intercept and β_1, β_2, β_3,…, β_i are the regression coefficients of explanatory variables X_1, X_2, X_3,…, X_i and ϵ_i is random error or disturbance term.

Statistical analysis

The data was tested for normality using Shapiro-Wilk test and the homogeneity of variance using Levine's test for Equality of Variances in order to satisfy analysis of variance (ANOVA) assumptions. Percentage data, such as survival rate was first transformed using natural log and the results were reported as geometric mean after taking antilog. Statistical Package for Social Scientists (SPSS) software Version 16 for Windows was used for data analysis. Data were presented as mean ± standard error (SE) and the graphs were drawn by Microsoft Office Excel 2010.

All growth parameters were subjected to ANOVA to compare the means among the treatments at 5% level of confidence. The significant differences between the means were separated using least significant difference (LSD).

RESULTS

Water quality parameters and ammonia

Results for water physico-chemical parameters are shown in Table 1. The results revealed significant differences (P<0.05) in dissolved oxygen concentration among the treatments. The highest dissolved oxygen (7.5 mg/L) was recorded in the tank where fish were fed on 30% CP treatment and the lowest (7.2 mg/L) was recorded in 45%. There were no significant differences (P>0.05) in the mean temperature among the treatments. pH ranged from 8.4 to 8.8 among the treatments.

Ammonia concentration exhibited significant (P<0.05) differences among the treatments with the highest observed in 45% CP. A polynomial regression equation y = 2E-05x^3 - 0.0017x^2 + 0.028x, R^2 = 0.96 (Figure 1) showed that ammonia concentration increased with increase in dietary protein levels. Furthermore, the coefficient of polynomial regression model (R^2) revealed that 96% of variation in ammonia concentration was due to different dietary protein levels. The study recorded ammonia concentration of 0.014 mg/L in 30%, 0.02 mg/L in 35%, 0.023 mg/L in 40% and significantly (P<0.05) higher 0.13 mg/L in 45% treatment.

Growth parameters

Higher significant (P<0.05) average weight gain (ADG), specific growth rate (SGR), survival percentage, protein index (PI), nitrogen metabolism (NM) and better feed conversion ratio were recorded in the tank where fish were fed on 40% CP, while the fish fed on 45% CP treatment recorded the lowest (Table 2).

Polynomial regression equation (Y = -0.005x^3 + 0.35x^2 - 4.67x, r^2 = 0.98) (Figure 2) showed that 40% dietary

Table 1. Water quality parameters measured in tanks stocked with *Tilapia rendalli* for 90 days (mean ±SE).

Parameter	30% CP	35% CP	40% CP	45% CP	P value
PH range	8.6-8.9	8.4-8.5	8.7-8.8	8.3-8.4	
DO(mg/l)	7.5±0.0.2^{d}	7.3±0.2^{b}	7.29±0.02^{b}	7.2±0.03^{a}	0.038
Temp (°C)	24.25±0.1^{a}	24.24±0.1^{a}	24.30±0.03^{a}	24.80±0.2^{a}	0.065
Ammonia (mg/L)	0.01±0.03^{a}	0.02±0.04^{b}	0.02±0.01^{b}	0.13±0.05^{d}	0.001

Values with the same superscript in a row are not significantly different (P>0.05).

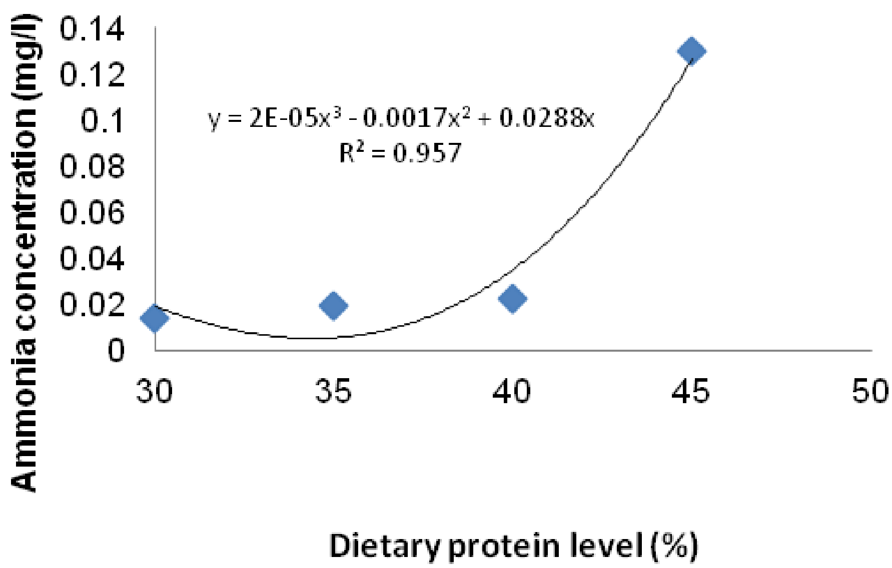

Figure 1. Relationship between ammonia concentration (mg/L) and dietary protein level for *Tilapia rendalli* raised in concrete tanks for 90 days.

Table 2. Growth and Feed conversion ratio of *Tilapia rendalli* raised in concrete tanks for 90 days (Results presented as mean ±SE).

Parameter	Treatments				
	30% CP	35% CP	40% CP	45% CP	P value
Initial weight (g)	9.77±0.02^{a}	9.87±0.2^{a}	9.53±0.23^{a}	9.7±0.23^{a}	0.783
Final weight (g)	46.6±0.49^{b}	50.08±0.9^{c}	53.93±0.93^{d}	42.1±0.23^{a}	0.001
Weight gain	36.9± 0.38^{b}	40.5±0.8^{c}	43 ±1.2^{d}	32.4±0.76^{a}	0.001
Feed conversion ratio	3.2±.0.03^{c}	3.01±0.05^{b}	2.9±0.05^{a}	3.5±0.062^{d}	0.001
Protein Efficiency ratio	0.7±0.04^{b}	0.77±0.09^{d}	0.72±0.1^{c}	0.5±0.062^{a}	0.001
Survival (%)	91±1.21^{a}	92±0.15^{b}	94±0.48^{d}	83±0.02^{c}	0.001
Condition factor (g/cm^{-3})	4.87±0.14^{c}	5.02±0.15^{d}	3.65±0.15^{b}	0.19±0.01^{a}	0.001
SGR (%day^{-1})	1.75±0.014^{b}	1.83±0.027^{c}	1.88±0.024^{d}	1.64±0.016^{a}	0.001
NM	894.97±0.3^{b}	977.1±0.21^{c}	1078.92±0.03^{d}	787.32±0.04^{a}	0.001
PI	0.36±0.08^{b}	0.41±0.05^{c}	0.46±0.02^{d}	0.33±0.06^{a}	0.001

Values with the same superscript in a row are not significantly different (P >0.05).

protein level was optimal. Additionally, the coefficient of polynomial regression model (R^2) explained that 98% of variation in final weight (g) was due to different dietary protein levels. Conversely, protein efficiency ratio (PER) and condition factor (CF) were the highest in 35% treatment and the lowest in 45% (Table 2).

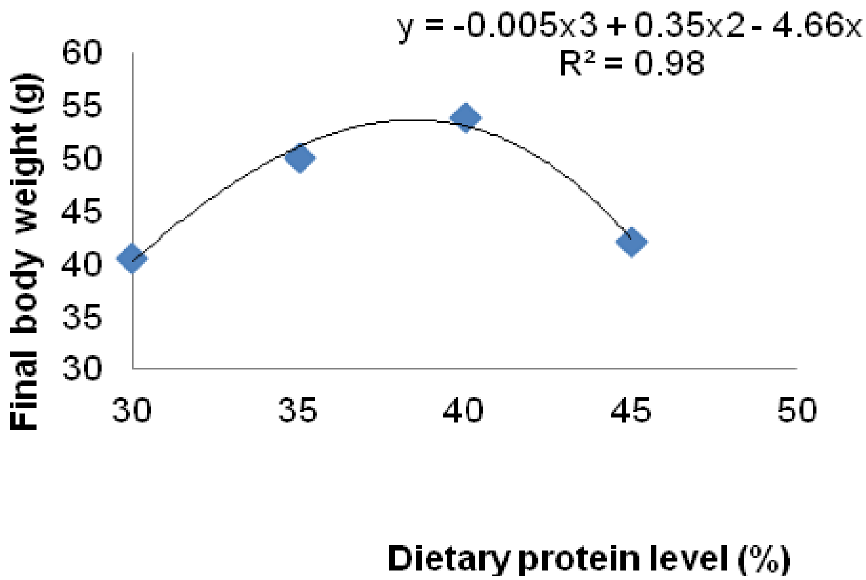

Figure 2. Relationship between final body weight and dietary protein level for *Tilapia rendalli* raised in concrete tanks for 90 days.

DISCUSSION

Water quality parameters, such as temperature, pH and dissolved oxygen (DO) were within the recommended range for the growth of most tilapias (Ross, 2000; Shepherd and Bromage, 1992). Ammonia concentration in 30, 35 and 40% treatments was within the recommended levels for optimum growth of tilapia (less than 0.05 mg/L) (Redner and Stickney, 1979). However, the ammonia level was the highest and above recommended value in 45% treatment. The highest ammonia concentration of 0.13 mg/L reported in 45% CP treatment could be attributed to endogenous ammonia which is related to higher quantity of nitrogen supplied by the 45% dietary protein levels (Thomas and Piedrahita, 1997).

Apparently, fish fed on 45% dietary protein may not have used all of the available protein, resulting into excessive ammonia accumulation in the tanks, as evidenced by lowest condition factor (0.19 g/cm^3) below recommended level which is above 1 g/cm^3 according to Mahomoud et al. (2011). These results agreed with Jindal et al. (2010) that excessive protein level in the diet increases nutrient retention in the culture systems. Furthermore, feed waste might have been as a result of poor assimilation. This suggests that at above 40% CP, the major portion (over 80%) of the nitrogen in the diet was added to the culture system, which ultimately polluted the environment. Lowest average weight gain (ADG), specific growth rate (SGR), protein index (PI) and nitrogen metabolism (NM) in the fish fed on 45% CP treatment could be due to the fact that highest ammonia level (0.13 mg/L) in 45% treatment might be toxic and eventually affecting growth of fish in the tanks as evidenced by low survival rate (83%) in 45% CP. Evidently, at high ammonia concentration (above 0.05 mg/L), metabolic rate is suppressed due to increased activity of glutamine synthesis, a detoxification mechanism (Mommsen et al., 1999). A glutamine synthesis is known to mediate the amination of glutamate and NH_3 in the formation of glutamine. Studies have shown that this pathway consumes vital brain ATP and glutamate resources, and can result in neurotransmission failure (Mommsen et al., 1999). This negatively affects growth performance. Poorer condition of the fish in the tanks was further evidenced by the lowest condition factor (0.19±0.01 g/cm^{-3}) observed in the fish fed on diet containing 45% treatment. The lowest condition factor indicated that the fish were affected by a number of factors, such as stress (Khallaf et al., 2003) attributed to high ammonia concentration.

In general, feed conversion ratio was reasonably high compared to the recommended rates (less than 2) in all treatments due to the fact that the feed might not have been palatable enough for the fish. Similar results were earlier reported for tilapia species (*Oreochromis niloticus*) by Abdel-Hakim et al. (2001). Nevertheless, feed conversion ratio (FCR) decreased with increasing dietary protein levels until the peak was reached at 40% CP and further increase in dietary protein levels resulted into decrease in FCR (Yang et al., 2002). Webster and Lim (2002) observed that better growth of fish relies on efficient synthesis of dietary protein into tissue protein.

The study further revealed that increase in the dietary protein level above 40% CP reduces protein efficiency ratio. Similar trend was earlier reported by Ahmad et al.

(2004) in tilapia species. The decrease in protein efficiency ratio at 45% dietary protein level might be attributed to the fact that beyond optimum point, more dietary protein is used as energy in the fish instead of being converted into muscle tissues (Kim et al., 1991).

The decrease in weight gain when feeding fish on the diet containing 45% dietary treatment could be due to the reduction in available energy for growth, because of inadequate non-protein energy necessary to deaminate and excrete excess absolute amino acids (Vergara et al., 1996). Too much protein in the diet may result into only a part being used to synthesise new proteins, while the rest is converted to energy (Alatise et al., 2006). When energy is in excess, fish may reduce feed intake thereby limiting the intake of amino acids needed for growth (NRC, 1993).

On the other hand, poor growth in the fish fed on 30% dietary treatment could be attributed to excessive carbohydrates in the diet which might have led to liver cell degeneration, hyperglycaemia and poor growth (Roberts, 1978; Halver, 1989). Furthermore, Makwinja et al. (2015) observed poor hepatosomatic index in the fish fed on 30% than the other treatments which suggested that low CP level in the diet correlate with reduction in available energy in the liver for growth.

Conclusion

This study has shown that in order to achieve acceptable fish growth, there is need to adopt optimal protein level of 40% CP. At 40% dietary protein, growth performance (weight gain, feed conversion ratio, specific growth rate) and water condition were within acceptable range for *T. rendalli* of the same size and resulted in high growth rate (1.88±0.024) compared to 30, 35 and 45% dietary protein levels. In conclusion, the suitability of 40% CP is in tank; however, studies should be conducted to investigate the suitability in other culture systems, such as earthen pond largely used by small scale farmers.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the Scottish Government through Dr. David Livingstone Scholarship Program for financial support for the study. Many thanks to the staff of the National Aquaculture Center for providing the facilities for conducting these experiments.

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