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The Effect of Ammonia on Growth and Survival Rate of Tilapia rendalli in Quail Manured Tanks

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Abstract This experiment was conducted to determine the effect of ammonia on growth and survival rate of Tilapia rendalli reared in concrete tanks for 14 weeks using quail manure. It was a completely randomized design (CRD) with five treatments; T1 (control, without quail manure), T2 (0.32kg quail manure/tank/week), T3 (0.40kg quail manure/tank/week), T4 (0.48kg quail manure/tank/week) and T5 (0.64kg quail manure/tank/week), replicated two times. Twenty-five fish, with an average individual initial weight of 14 g were stocked per tank, each measuring 2m × 2m (4m²). Every fortnight individual fish were weighed on a sensitive scale and the length measured on a measuring board to the nearest 0.1 cm. The results of the experiment indicated that fish raised under treatment 4 (T4) grew much faster (ANOVA, P<0.05) than the other groups with weight gain of 246%. T2 had the highest weight gain of 229%. Every fish in all treatments was measured for size. The results of the experiment indicated that fish raised under treatment 4 (T4) grew much faster (ANOVA, P<0.05) than the other groups with weight gain of 246%. T2 had the lowest mean weight gain of 86.08%. The final mean weights ranged from 26.23 g to 47.55 g, while total length ranged from 111mm to 140.1 mm ± 2.29mm respectively. The study further revealed that in order to achieve table size growth and survival of fish, 0.48 kg quail manure/week/4m² should be used as optimum for better growth of fish, although no primary productivity was measured to estimate zooplankton biomass. At the same time, daily growth rate was good and within acceptable range for fish of the same size. Survival rate in all treatments was very high, with the lowest being above 60%. The study further demonstrated that higher amounts of ammonia affected growth and survival of the fish in treatment 5 (T5), a tank with the highest amount of quail manure added per week.

Keywords Ammonia; Effect; Growth; Survival rate; Quail manure; Concrete tanks

Introduction There has been tremendous development in the farming of tilapia worldwide, in which the commodity is not only the second most important farmed fish globally, next to carps but is also described as the most important aquaculture species of the 21st century (Shelton, 2002). The main purpose of fish culture is to raise the fish to table size in the shortest possible time (Lovell, 1989). Tilapia, especially Oreochromis niloticus (Nile tilapia) is reported to be an appropriate species in aquaculture owing to its wide adaptability, and its ability to convert many organic, animal and agriculture wastes into high quality protein (Balarin, 1979). However, the cost of feed still remains high and feed costs make up the largest cost of fish production, hence a challenge to most fish farmers (Steffens, 1989). In all regions of the world, the increase in the cost of raw ingredients for manufactured or on-farm aquafeeds resulted in an increase in aquafeed prices from 20 to 40 percent, thus forcing farmers to adopt alternative strategies to secure feeds (Rana Sunil Siriwardena, and Hasan, 2009). In the light of such price increases, farmers are increasingly looking for alternative sources of feeds such as trash fish, animal by-products and grain by-products, or are reverting to the use of single ingredient supplementary feeding regimes, reduced feeding frequency and ration. These types of interventions to mitigate against rising feed costs will compromise fish growth, health and welfare and could reduce fish productivity and production (Rana Sunil Siriwardena, and Hasan, 2009). The fish feed plays an important role in the value chain as it implies important control of the quality of raw materials, which is crucial for the food safety as well as efficient high quality feed types that ensures optimal growth for different fish species farmed under a variety of different conditions (Thorarinsdottir et al., 2011). The feed cost has to be controlled as this is most often approximately 50% of the total production cost in
aquaculture (Thorarinsdottir et al., 2011). In recent years most farmers have engaged in the integration of fish and quail rearing. The use of quail manure as a fertilizer to enhance the growth of phytoplankton in ponds raises a lot of concerns due to ammonia in quail manure which could potentially affect the growth and survival rate of fish, (Jobling, 1994; Durborow et al., 1997). Ammonia toxicity to fish depends on the concentration of unionized ammonia (NH$_3$) (Handy & Poxton, 1993). Percent NH$_3$ increases with temperature and pH. Both temperature and pH increase the NH$_3$ concentration (Nettena et al, 2013). In general, more NH$_3$ and greater toxicity exists at higher pH, which implies that NH$_3$ is more dependent of pH than temperature. This is because the percent of toxic un-ionized NH$_3$ increases with pH (Frank, 1998). NH$_3$ toxicity will become more pronounced under higher temperatures, but that effect on aquatic macrophytes will strongly depend on pH of the water layer and specific metabolic adaptations of different species (Nettena et al, 2013). However, less concentration of this NH$_3$ can be tolerated at lower pH (Frank, 1998).

Materials and Methods

The experiment was conducted in duplicates using 10 × 2m × 2m (4m$^2$) concrete tanks at The National Aquaculture Development and Research Centre (NARDC) in Mwekera for a period of 14 weeks. The ponds were supplied with borehole water that was maintained at constant level throughout the experimental period. Twenty-five fish (Tilapia rendalli), with an average individual initial weight of 14 g and 79.49 mm length, were stocked per tank. Tilapia rendalli fingerlings that were used in the experiment were collected from a 150 m$^2$ hatchery earthen pond located within the fish farm. Fingerlings were acclimatized for a period of one week before the commencement of the experiment. During the acclimatizing period, the fingerlings were fed on maize bran at 4% of their body weight.

Key water quality parameters such as water temperature, pH and dissolved oxygen (DO) were measured twice per day (at 09:00hrs and 15:00hrs), using a Horiba U-10 water quality checker. The checker sensor measures by being directly submersed or dipping the probe 20 cm into the water. Total ammonia-nitrogen was monitored twice a week using standard methods (Tucker, 1993; APHA, 1999).

Every fortnight individual fish were weighed on a sensitive scale and the length measured on a measuring board to the nearest 0.1 cm. For accurate results the fish were starved for 24 hours prior to weighing. The growth of fish was compared in tanks with different amounts of quail manure. Initial and final weight, percent in weight gain, increase in total length, and coefficient of the final weight of individual and condition factor were analyzed using analysis of variance (ANOVA).

The growth parameters were calculated using the following formulae:

Conditional factor (K): The formula is of the form: 
\[
K = \frac{W}{L^3}
\]

Where K = Fulton’s condition factor, W = the weight of the fish, and L is the length (usually total length).

Specific growth rate (SGR), expressed as percent body weight per day, was calculated from: 
\[
SGR = 100 \left( \frac{\ln W_f - \ln W_i}{t} \right)
\]

Where:-

W$ _f$ = final mean weight,
W$_i$ = initial mean weight, and
t = experimental time in days

Percentage increase in weight (IWG %) was computed following the formula provided by De Silva and Anderson (1995): 
\[
%IWG = \left( \frac{W_f - W_i}{W_i} \right) \times 100
\]

The experiment was a completely randomized design (CRD) with five treatments; T1 (control, without quail manure), T2 (0.32kg quail manure/tank/week), T3 (0.40kg quail manure/ tank/week), T4 (0.48kg quail manure/tank/week) and T5 (0.64kg quail manure/tank/ week), replicated two times.

Statistical package for social sciences (SPSS) version 12.0 software was used in the statistical analysis and variables differences were considered significant at P < 0.05. One–way analysis of variance (ANOVA) was used to test the differences between treatments during the experiment.

Results

As shown in Figure 1, there were differences in the final mean weight gain among the treatment groups.
Fish in treatment 4 (T4), with 0.48kg quail manure showed the best (P<0.05) growth performance (Figure 1), with the final average body weight of 47.55g. Slow and inconsistent growth was however, observed in treatments 5 (T5).

Growth trends of Tilapia rendalli supplied with different rate of quail manure are shown in Fig.1. The significant (P<0.05) growth of fish was influenced by the Quail manure application rates at all water temperature. All the treatments showed fast and constant growth during the onset of the experimental period except T5, which had the highest amount of quail manure. Furthermore, after the third week, a pronounced difference in growth rate of fish was observed between the treatment groups and this difference was significant (ANOVA, P<0.05) (Fig. 1). However, T1 (control), recorded steady and constant growth throughout the experimental period.

Meanwhile, Table 1 shows the mean final body weight of fish that varied from 26.23g to 47.55g with highly significant differences between treatments (p<0.05).

The study showed that Treatments 5 (T5) and 4 (T4) were significantly different from each other while others were not. Final total length ranged from 111mm± 2.29mm to 140.1mm ± 2.29mm respectively. Survival rate in all treatments was very high, with the lowest being above 60% (Table 1). The key water quality parameters such as pH, DO, Temperature and Ammonia were not significantly different among the treatment groups (ANOVA, P > 0.05, Table 2).

The study showed that Treatments 5 (T5) and 4 (T4) were significantly different from each other while others were not. Final total length ranged from 111mm± 2.29mm to 140.1mm ± 2.29mm respectively. Survival rate in all treatments was very high, with the lowest being above 60% (Table 1). The key water quality parameters such as pH, DO, Temperature and Ammonia were not significantly different among the treatment groups (ANOVA, P > 0.05, Table 2).

Table 1 Growth performance of T. rendalli raised under fertilized ponds for 14 weeks

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>QM application rate (kg/wk/2 m²)</td>
<td>0.00</td>
<td>0.32</td>
<td>0.40</td>
<td>0.48</td>
<td>0.64</td>
</tr>
<tr>
<td>Initial mean weight ± SE (g)</td>
<td>12.704±0.37</td>
<td>14.096±0.06</td>
<td>13.188±0.12</td>
<td>13.744±0.18</td>
<td>15.192±0.14</td>
</tr>
<tr>
<td>Final mean weight ± SE (g)</td>
<td>29.06±1.66a</td>
<td>26.23±1.62a</td>
<td>32.45±1.27a</td>
<td>47.55±2.7b</td>
<td>31.4±3.34c</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>16.356</td>
<td>12.134</td>
<td>19.262</td>
<td>33.806</td>
<td>16.208</td>
</tr>
<tr>
<td>daily growth rate (g day⁻¹)</td>
<td>0.17</td>
<td>0.12</td>
<td>0.2</td>
<td>0.34</td>
<td>0.17</td>
</tr>
<tr>
<td>Weight gain (%)</td>
<td>128.75</td>
<td>86.08</td>
<td>146.06</td>
<td>245.97</td>
<td>106.69</td>
</tr>
<tr>
<td>SGR%/ day</td>
<td>0.844</td>
<td>0.634</td>
<td>0.919</td>
<td>1.267</td>
<td>0.741</td>
</tr>
<tr>
<td>Initial SL (mm)</td>
<td>56.36</td>
<td>60.92</td>
<td>57.8</td>
<td>60.84</td>
<td>63.68</td>
</tr>
<tr>
<td>Final SL (mm)</td>
<td>89.5</td>
<td>86.4</td>
<td>89.8</td>
<td>110.4</td>
<td>91.8</td>
</tr>
<tr>
<td>Initial TL (mm)</td>
<td>75.16</td>
<td>77.44</td>
<td>75.76</td>
<td>79.08</td>
<td>82.08</td>
</tr>
<tr>
<td>Final TL (mm)</td>
<td>118.5</td>
<td>111</td>
<td>117.3</td>
<td>140.1</td>
<td>122.5</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>85.28</td>
<td>96.83</td>
<td>89.22</td>
<td>95.44</td>
<td>60.67</td>
</tr>
</tbody>
</table>

Note: *Value are means ± s.e. means within same column not sharing a common superscript are significantly different (p<0.05)

Table 2 Water quality parameters during 14 weeks experimental period (Mean ±SE)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water Temp.(°C)</th>
<th>DO(mg L⁻¹)</th>
<th>pH</th>
<th>'TAN'</th>
<th>Unionized ammonia (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.81±0.84</td>
<td>5.98±0.33</td>
<td>7.67±0.12</td>
<td>0.32±0.31</td>
<td>0.0057</td>
</tr>
<tr>
<td>2</td>
<td>24.11±0.87</td>
<td>6.13±0.36</td>
<td>7.71±0.13</td>
<td>0.467±0.29</td>
<td>0.0084</td>
</tr>
<tr>
<td>3</td>
<td>24.13±0.87</td>
<td>6.03±0.28</td>
<td>7.81±0.14</td>
<td>1.03±0.02</td>
<td>0.0184</td>
</tr>
<tr>
<td>4</td>
<td>23.91±0.87</td>
<td>6.02±0.31</td>
<td>7.33±0.14</td>
<td>1.30±0.13</td>
<td>0.0233</td>
</tr>
<tr>
<td>5</td>
<td>24.27±0.86</td>
<td>6.12±0.31</td>
<td>7.30±0.16</td>
<td>1.98±1.25</td>
<td>0.0354</td>
</tr>
</tbody>
</table>

Note: *TAN = Total ammonia-nitrogen in mg L⁻¹
The average water temperature was 22.44 °C, while pH varied between 7.30±0.12 and 7.81±0.16 across all the treatments. The minimum and maximum DO concentration varied between 5.98±0.33 mg L⁻¹ and 6.13±0.36 mg L⁻¹ across all treatments, respectively. The unionized ammonia varied between 0.0057 mg L⁻¹ and 0.0354 mg L⁻¹.

Discussion
From the results presented above, there were differences in the growth of fish in all the treatments. The differences in growth and survival were mainly due to the different levels of ammonia in the water. Rapid and slow growth was observed in T4 (0.48 kg quail manure/week) and 5 (0.64 kg quail manure/week) respectively. The study demonstrated that higher amounts of ammonia affected growth and survival of the fish in treatment 5 (T5), a tank with the highest amount of quail manure added per week. However, T1, T2 and T3, showed constant growth till the end of the experimental period, with no significant differences observed among them (Fig. 1). The growth rate of fish in fertilized tanks was partly attributed to the leftover feed from the quails and the fertilization of the water which stimulated the growth of phytoplankton and zooplankton. In order to reduce expenses on commercial fish feed, supplementary ingredients in use among the farmers have also included: soybeans, maize bran, other various plant proteins, insects and algae. These have grown fish to attain table size of between 100 and 350g. However, it must be emphasized that consumer preference in Zambia differs a lot and size of fish on the market does not matter much.

Results of the experiment showed that in order to achieve acceptable fish growth and survival of fish, 0.48 kg quail manure/week/2m² should be used as optimum for better growth of fish, although no primary productivity was measured to estimate zooplankton biomass in this study. At that level, growth performance was good and other indicators were well within acceptable range of fish of the same size. Similarly, Nile tilapia (O. niloticus) fingerlings averaging 19.0± 1.0 g in weight were reared at four different concentrations of UIA-N (0.01, 0.05, 0.1 and 0.15 mg/l) besides the control using 15 glass aquaria (40 x70 x60 cm) (El-Sherif, and El-Feky, 2008). At the end of the experimental period (after 75 days from the stocking) the final average body weights (FBW) of Nile tilapia (O. niloticus) fingerlings were 37.2, 36.4, 32.5, 26.9 and 37.7 g for UIA-N concentrations of 0.01, 0.05, 0.1, 0.15 and 0.004 mg/l, respectively (El-Sherif, and El-Feky, 2008).

In this study, all treatments, except 5, recorded high survival rate (Table 1), while El-Sherif, and El-Feky (2008) reported that no mortality occurred in any of their experimental groups throughout the experimental period. The use of quail manure which was rich in proteins underwent decomposition and the end product was ammonia which contained unionized ammonia. Ammonia exists in two forms: unionised ammonia (NH3-N), and ionised ammonia (NH4+), the sum of these two is called total ammonia nitrogen (TAN) (Molleda, et al., 2007). The relative concentration of ammonia is primarily a function of water pH, salinity and temperature (Pillay and Kutty 2005). The higher toxicity levels of NH3-N and CO2 in water depends on the water’s pH controls acid-base equilibrium; as an example, at 20°C and a pH of 7.0, the mole fraction of NH3-N is 0.004, but at a pH of 10, the NH3-N increase to 0.8 at the same temperature (Timmons et al., 2002).

Based on the results obtained, the ammonia level was highest in T5, which could have been toxic, eventually affecting growth and survival rate of fish in the tank. Higher mortalities in T5 could equally, be attributed to the high amount of ammonia levels recorded, although the values were below unionized Ammonia of 0.05 mg L⁻¹ that may harm fish. Normally, warm water fish are more tolerant to ammonia toxicity than coldwater fish, and freshwater fish are more tolerant than saltwater fish, so in general, NH3-N concentrations should be held below 0.05 mg L⁻¹ and TAN concentrations below 1.0 mg L⁻¹ for long-term exposure (Timmons et al., 2002). During the day especially after adding quail manure some fish would be seen coming to the surface gasping for air, showing signs of loss of appetite and red streaking on the fins, gills turning red at the same time fish appearing lethargic. These results were in line with a study conducted by Ogbonna and Chinomso (2010) on Ammonia poisoning and it was concluded that ammonia poisoning could happen suddenly, or over a period of days. Similarly, a study...
by EL-Shebly and Gad (2011), indicated that the fish exposed to increased concentrations of ammonia showed the following signs: moved very rapidly, lost equilibrium in water, increase in their movements, ventilation, convulsions, spiral swimming, efforts to swallow air from the surface of water, increase in mucous secretion in the gills and on the body surface, hemorrhage in the gills and darkening on the skin.

Ogbonna and Chinomso (2010) reported that in order to ascertain the cause of the death of these fishes on a fish farm, some selected physicochemical and microbiological parameters were used to determine the concentration which resulted in lethal effect on the fishes. Test results, therefore, confirmed that ammonia concentration of above 0.20 mg L\(^{-1}\) in fish ponds had a tendency to harm the fishes. That could partly be an explanation of why mortalities were recorded in T5 which was closer to the lethal amount of 0.05 mg L\(^{-1}\). That amount of ammonia equally, explained the slow growth rate which was recorded in Treatment 5. According to Durborow et al. (1997), ammonia poisoning occurred most often when there was less or no circulation of water in the pond. Fish size was equally affected by ammonia. Smaller sized fish exposed to a higher dosage per body weight unit become more susceptible to unionized ammonia (Piedras et al., 2006). Correspondingly, Cavero et al. (2004) exposed young Arapaima gigas to a concentration of 25 mg/L NH\(_3\)+ NH\(_4\)\(^+\) or 2 mg/L NH\(_3\) for 24 hours and no effect was observed. Survival of fish was more critical mostly during the period just after stocking because by then the fish would have been stressed but had to acclimatize to the new environment. Other than handling, stress can also be caused by the differences in temperature, which could be worsened if the amount of toxic ammonia in the pond was very high as observed in the present study. In the same vein, we could not rule out the low oxygen uptake by fish. According to Mallya and Thorarensen (2007), it was commonly thought that if there was not enough oxygen in the water, then the fish would be seen gasping at the surface but this was a last resort means to breathe. The authors reported that the first indication there may be a dissolved oxygen problem in the water was when the fish become unusually lethargic and stop feeding. As oxygen levels decrease, the fish do not have enough energy to swim and feeding utilises yet more oxygen (Mallya and Thorarensen, 2007).

Water quality parameters remained within the acceptable limits for tilapia growth as described by Boyd (1984). Most of the water quality parameters (DO, pH and temperature) were not significantly different in all the treatment groups except total ammonia-nitrogen. Shepherd and Bromage (1992) reported that carp, catfish and tilapia could withstand Dissolved Oxygen levels of below 2 mg/L provided it was for short periods. Lawson (1994) however, observed that to support life for several hours, a minimum of 1.0 mg/L was required while 1.5 mg/L was necessary to support fish for several days. Tilapia routinely survived dawn dissolved oxygen concentrations of less than 0.5mg/L, considerably below the tolerance limits for other cultured fish. The other minimum water quality parameters reported by Lawson (1994) were as follows: Total ammonia-nitrogen (TAN) less than 1.0 mg/L, Nitrite-nitrogen 0.1mg/L in soft water. The author stated that massive mortality of tilapia occurred within a couple of days at un-ionized ammonia >2 mg/L. Prolonged exposure to un-ionized ammonia levels greater than 1 mg/L causes losses, especially among fry and juveniles in water with low DO (Lawson, 1994).

**Conclusion**

The study has highlighted the effects of quail manure on growth and survival rate of the fish. The growth rate of fish in fertilized tanks was partly attributed to the leftover feed from the quails and the fertilization of the water which stimulated the growth of phytoplankton and zooplankton. Results of the experiment showed that in order to achieve acceptable fish growth and survival of fish, 0.48 kg quail manure/week/2m\(^2\) should be used as optimum for better growth of fish, although no primary productivity was measured to estimate zooplankton biomass in this study. At that level, growth performance was good and other indicators were well within acceptable range of fish of the same size. The study further demonstrated that higher amounts of ammonia affected growth and survival of the fish in treatment 5 (T5), a tank with the highest amount of quail manure added per week.

**Acknowledgement**

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