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Effect Of Stocking Density On Survival And Body Composition Of Nile Tilapia (*Oreochromis Niloticus*) Fed MULTI Feed And NIOMR Feed In Semi Flow-Through Culture System.

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Abstract

This study was carried out to investigate the growth response and body composition of Nile tilapia; *O. niloticus* to two feed type: MULTI feed (foreign) and NIOMR feed (local) of 32% and 44% crude protein (CP) respectively at three stocking densities 158, 237 and 316/m^3 in semi-flow through culture system. The experiments were conducted in two phases in succession using twelve (12) circular fibre glass tanks stocked with mixed sex fingerlings of Nile tilapia of average weight of 6.8g at the three stocking densities and cultured for a period of 24 weeks. The result of the study showed significantly ($P < 0.05$) highest growth, feed conversion and survival rate in treatment II (MULTI feed at 158 fish/m^3) while significantly lowest growth rate and feed conversion was recorded in treatment V. The lowest survival rate (94.02%) was recorded in treatment VI. In terms of the proximate composition of the Nile tilapia fed the experimental diets; treatments that were fed with Multi feed (II, IV, and VI) showed higher values than fish fed with NIOMR diet (I, III, and V) at equivalent stocking densities at the end of the trial. All water quality parameters are within the acceptable and suitable range for proper growth of *O. niloticus.* Based on the overall growth performance; treatment II at stocking density of 158 fish/m^3 fed Multi feed is recommended.

Keywords: Nile tilapia, stocking density, growth performance, body composition, flow-through system.

1. INTRODUCTION

The production of farmed tilapia is among the fastest expanding food sectors in the world. Nile tilapia (*Oreochromis niloticus*) is the most cultured fresh water species among the farmed tilapia and contributes about 71% of the world total tilapia production (FAO, 2002). Tilapia 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). It represents the species of choice due to its high growth rate, significant tolerance to environmental stress, ease of reproduction, and high market demand (El-Sayed, 2006).

Addition of artificial feeds plays an important role especially under conditions of heavy stocking, when natural feed supply has declined or completely disappeared. The added feeds should be rich in protein, carbohydrate and fats, and should also contain vitamins, minerals and growth-promoting substances to be physiologically balanced (Huisman *et al.,* 1979). Fish malnutrition would reduce growth performance and may cause disease or even death (Lovell, 1989). Therefore, it is essential to develop suitable feeds to be used either as a supplementary diet in pond or as a complete diet in tanks. Intensive culture of tilapia in tanks has been globally expanding (El – Sayed, 2006). Several factors such as savings in labour and easier stock management are the main reasons for intensification of fish farming in concrete or fibreglass tanks in many countries (El-Sayed and Gaber, 2005). Tanks vary in size, shape and composition. The preferred shape is circular with sloping bottoms and central drainage. Concrete and fibreglass are the most popular materials although plastic, metal, and poly-board are also used. Intensification of tilapia culture is a good solution for increasing fish production, and to optimize fish intensification, both feed quality and stocking density should be considered. Therefore, the present study was conducted to investigate the effect of rearing density and two feed type {MULTI feed (foreign) and NIOMR feed (local)} and their interaction on growth characteristics and body composition of Nile tilapia.

2. MATERIAL AND METHODS

2.1 Study Area:

The study was conducted at the Nigerian Institute for Oceanography and Marine Research, Sapele Out-Station (N05^{0}54'03.5"E05^{0}39'56.4"). The experiment was conducted in two phases in succession using twelve (12) circular fibreglass tanks each with a capacity of 3.08m^3 of water. The tanks were mounted indoor in a flow through and arranged in a row. The twelve (12) circular fibreglass tanks used in this experiment were identical in

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shape and size. The floor of each tank drained to the centre. Drainage of the tank was on the outside via 100mm PVC pipes and gate valves. Each tank received continuous flow through with water 6 – 8 hours per day from a borehole passing through a water treatment plant to correct the pH. Each tank was washed, cleaned, and disinfected with sodium chloride (NaCl) after which the tanks were filled with water to a depth of 60.5 cm and allowed to settle for a day before introducing the fish.

Each tank was stocked with fingerlings of mixed sex of Nile tilapia (*O.niloticus*) of average weight of 6-8g; sourced from the Outstation farm of Nigerian Institute for Oceanography and Marine Research, Sapele, Delta State, Nigeria and cultured for a period of 24 weeks in each phase. Different stocking densities were used (300, 450 80 and 600 fish per tank), and the trial was repeated twice. The stocking density of 300, 81 400, 600 fish per tank translated to 158, 237, and 316 m^{-3}. The tanks were drained daily by opening the gate valve and allowing solids (uneaten feed and faeces) that settled at the bottom to be flushed out and an equal amount of water was introduced at the same rate to replace the quantity that was drained thereby maintaining the original volume of water in the tank.

Two types of feed were used to feed the fish. These were the MULTI feed (foreign) and NIOMR feed (local). The daily feeding rate for the first week was 5% of the total stocking biomass. Thereafter, fish were sampled every two weeks to obtain information for adjustment of feeding rates. Every two weeks thereafter tanks samples provided information for the adjustment of feeding rates passed on new tilapia biomass in each experimental tank. Fish were fed with 44% Crude Protein (NIOMR diet) and 32% protein (MULTI feed) twice daily at 0800 hour and 1600 hour. Six experimental treatments were used (see Table 1). Each combination was replicated twice in two phases. The experimental design was two feed type x 3 stocking densities replicated twice. A total of 12 culture units were used in the two phases.

2.2 Proximate Analysis:

Proximate analysis of the experimental diets and fish was carried out.

2.3 Physio-chemical parameters:

Sampling of the cultured fish was carried out bi-weekly for a period of 24 weeks for the collection of data to determine the variation among the treatments. Throughout the entire culture period different water quality parameters like temperature, dissolved oxygen, pH, nitrate, nitrite and total ammonia were regularly monitored.

2.4 Statistical Analysis:

Data collected were subjected to statistical test using analysis of variance (ANOVA). Mean separation was done using Duncan Multiple Range Test and Least Significant Difference. All tests were carried out at 5% probability level (P =.05). The Genstat Statistical Package (version 8.1) was used for the analysis.

3. RESULTS

Table 1. Feed types/stocking densities

FEED TYPE	TREATMENTS	No. fish/m^3
NIOMR feed (Local) 44% CP	I	158
	III	237
	V	316
Multi feed (Foreign) 32% CP	II	158
	IV	237
	VI	316

Key:

I = NIOMR feed @ 158 fish/m^3

II = MULTI feed @ 158 fish/m^3

III = NIOMR feed @ 237 fish/m^3

IV = MULTI feed @ 237 fish/m^3

V = NIOMR feed @ 316 fish/m^3

VI = MULTI feed @ 316 fish/m^3

3.1 Proximate Analysis of Feeds:

The data for the proximate analysis of the tested diet (on dry matter basis) is presented in Table 2. The values for crude protein, ether extract and crude fibre were higher in NIOMR diet than in MULTI feed while ash content was the reverse. The different growth parameters {final mean weight, mean weight gain, specific growth rate (SGR) of Nile tilapia fed with NIOMR feed and MULTI feed at different stocking densities are shown in Table 3}. At the end of the experiment (24 weeks), the different growth parameters were significantly affected by stocking densities (P = .05) among the treatments. There was also a significant difference (P = .05) between the two different types of diet even at similar densities. However, there were no significant differences in specific growth rate between the NIOMR and MULTI feed diet in all the treatments.

Table 2. Proximate composition of NIOMR feed and Multi feed.

NIOMR FEED		**MULTI FEED**
Ingredients (%)		Ingredients (%)
Crude protein	44.38	32.00
Ether extract	5.23	4.00
Crude fibre	5.60	5.00
Ash	4.33	8.00
Moisture	11.60	-
NFE	28.60	-
P	-	1.20
Ca	1.20	-

3.2 Growth performance of O. niloticus fed NIOMR feed and MULTI feed in a semi flow-through culture system.

There was significant increase in fish weight gain over the culture period (P=.05) as shown in Table 3. Fish in treatment II showed the highest mean weight gain of 120 97.03g which was significantly different from all the other treatments as indicated in Table 3. Fish in treatment III and treatment VI recorded 71.72g and 71.16g respectively, which were not significantly different from each other. The least weight gain of 59.20 g was observed in Treatment V.

The average values of Specific Growth Rate (SGR) ranged between 1.46 in treatment I and V to 1.64 in treatment II (see Table 3). However, the differences in SGR values between the different experimental treatments were not significant (P =.05). Table 3 shows that there were significant differences in final mean weight between the two feed types and among the three stocking densities. The highest final mean weight of 103.68g was recorded in treatment II that was fed with Multi feed at a stocking density of 158 fish/m^3 while the least final mean weight of 65.34g was observed in treatment V fed with NIOMR diet and stocked at 316 fish/m^3. In general, final mean weight increased with decreasing stocking density and treatments that were fed with Multi feed performed better than those fed with NIOMR diet at equivalent stocking densities.

Feed intake increased progressively from a low intake of 510.39g for treatment I to a high feed intake of 1034.88g for treatment VI (Table. 3). Furthermore, the data indicate that at a similar stocking density fish fed with NIOMR diet consumed significantly less feed than those fed with Multi feed diet.

Feed conversion ratio varied significantly among the treatments (Table 3). Treatments I and II, recorded the lowest FCR which were not significantly different from each other. In a similar manner, treatments III and IV performed equally and recorded intermediate FCR while treatments V and VI, stocked at the same rate but received two different feed types had the highest FCR and were not significantly different from each other. Thus, the FCR increased with increasing stocking density for the two feed types and better at lower stocking densities.

Survival rate exceeded 90% in all treatments (Table 3) but there were significant differences among treatments. The highest survival rate of 98.0% was recorded in treatment II while the lowest rate of 94.02% was recorded in treatment VI. There was no significant difference in survival rate among treatments I, III, IV and V.

Table 3: Growth performance of *O. niloticus* fed NIOMR feed and MULTI feed in a semi flow-through culture system.

PARAMETERS	TREATMENTS					
	I	II	III	IV	V	VI
Initial mean weight (g)	6.92^{a}	6.65^{a}	5.78^{c}	6.85^{a}	6.14^{b}	5.83^{c}
Mean Weight gain (g)	73.93^{d}	97.03^{a}	71.72^{c}	86.81^{b}	65.34^{f}	76.99^{c}
Final mean weight (g)	80.85^{d}	103.68^{a}	77.5^{e}	86.81^{b}	65.34^{f}	76.99^{c}
Specific Growth Rate (%/day)	1.46^{a}	1.64^{a}	1.55^{a}	1.56^{a}	1.46^{a}	1.58^{a}
Survival rate (%)	96.33^{b}	98.0^{a}	96.96^{b}	97.0^{b}	96.98^{b}	94.02^{c}
Feed intake (g feed/fish)	510.39^{f}	633.01^{e}	749.98^{d}	865.44^{c}	887.97^{b}	1034.88^{a}
Feed conversion Ratio	6.90^{c}	6.52^{c}	10.46^{b}	9.97^{b}	13.59^{a}	13.44^{a}

Means with different superscripts in the same row are significantly different (P =.05)

NOTE:

I = NIOMR feed @ 158 fish/m^3

II = MULTI feed @ 158 fish/m^3

III = NIOMR feed @ 237 fish/m^3

IV = MULTI feed @ 237 fish/m^3

V = NIOMR feed @ 316 fish/m^3

VI = MULTI feed @ 316 fish/m^3

3.3 Proximate analysis of Nile tilapia:

The proximate analysis/composition of the Nile tilapia fed the experimental diets is indicated in Table 4. There was significant difference in Dry Matter composition (DM) of fish fed the two feed types and among the three stocking densities. The highest DM composition of 23.21 was recorded in treatment II while the least DM of 20.24 was observed in treatment III. In general the treatments that were fed with Multi feed (II, IV, and VI) showed higher values than fish fed with NIOMR diet (I, III, and V) at equivalent stocking densities. Values for all treatment exceeded the value of 20.05% recorded at the beginning of the treatment. There was a significant difference between treatment I and all the other treatments (II, III, IV, V, and VI) which were similar; the least protein value of 60.85% was in treatment I. The values for all treatments were higher than the 56.60% recorded for fish at the beginning of the experiment (Table 4). There was no significant difference in the ether extract among all the treatments (P=.05). The highest ether extract of 21.89 was recorded in treatment II while the least ether extract of 21.10 was observed in treatment I. In general, treatments that were fed with Multi feed showed higher values than those fed with NIOMR diet at equivalent stocking densities. The values exceeded the value obtained at the beginning of the experiment (see Table 4). The ash composition varied significantly among the treatments as indicated in Table 4. Treatment I and II stocked at the same density but received two different feed types recorded a lower ash content which was not significantly different from each other. Treatments I and II that represented the low density recorded the lowest values of 16.27% and 16.40% respectively which were not significantly different from each other (see Table 4). However, at the intermediate and high densities, the values increased but were not significantly different from each other.

Table 4: Proximate analysis/composition of Nile tilapia (*O. niloticus*) fed the experimental diets.

PARAMETERS (%)	TREATMENTS						
	Initial Status	**I**	**II**	**III**	**IV**	**V**	**VI**
Dry Matter	20.05	22.04^{b}	23.21^{a}	20.24^{c}	23.20^{a}	22.10^{b}	23.20^{a}
Crude Protein	56.60	60.85^{b}	61.74^{a}	61.53^{a}	61.94^{a}	61.74^{a}	61.60^{a}
Ether Extract	18.15	21.10^{a}	21.89^{a}	21.39^{a}	21.36^{a}	21.39^{a}	21.53^{a}
Ash	23.10	16.27^{b}	16.40^{b}	17.19^{a}	17.84^{a}	17.80^{a}	17.82^{a}

Means in same row with different superscripts are significantly different ($P < 0.05$).

Key:

I = NIOMR feed @ 158 fish/m^3
II = MULTI feed @ 158 fish/m^3
III = NIOMR feed @ 237 fish/m^3
IV = MULTI feed @ 237 fish/m^3
V = NIOMR feed @ 316 fish/m^3
VI = MULTI feed @ 316 fish/m^3

3.4 Water Quality Analysis:

Table 5 shows the result of the water quality analysis. The results show that the highest temperature of 25.80^0C was recorded for treatments I, IV and VI while the least temperature of 25.50^0C was observed in treatment III and V. The highest pH of 7.00^0C was recorded for treatment III and IV while the least pH of 6.98^0C was recorded in treatments I, V and VI. The highest Dissolved oxygen (DO) of 11.05mg/L was recorded for treatments III while the least DO of 10.93mg/L was recorded in treatment IV. The highest Ammonia nitrogen (NH3N) of 3.00mg/L was recorded for treatment VI while the least Ammonia nitrogen (NH3N) of 2.43mg/L was recorded in treatment II. The highest unionized ammonia (NH3) of 0.02mg/L was recorded for treatments I, III, IV, V and VI while the least unionized ammonia (NH3) of 0.01mg/L was recorded in treatment II. In general, there was no significant difference in the water quality parameters recorded among the treatments. All water quality parameters are within the acceptable and suitable range for proper growth of *O. niloticus.*

Table 5. Water quality parameters during the experimental period.

PARAMETERS	TREATMENTS					
	I	**II**	**III**	**IV**	**V**	**VI**
Temperature ^{0}C	25.80^{a}	25.60^{a}	25.50^{a}	25.80^{a}	25.50^{a}	25.80^{a}a
pH	6.98^{a}	6.99^{a}	7.00^{a}	7.00^{a}	6.98^{a}	6.98^{a}
Dissolved Oxygen (mg/L)	10.95^{a}	10.94^{a}	11.05^{a}	10.93^{a}	11.02^{a}	10.94^{a}
Ammonia Nitrogen (NH_3N)	2.46^{a}	2.43^{a}	2.69^{b}	$2.72^{\mathbf{b}}$	2.88^{b}	3.00^{c}
Unionized ammonia (NH_3)	0.02^{a}	0.01^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{a}

Means in same row with different superscripts are significantly different ($P < 0.05$).

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VI = MULTI feed @ 316 fish/m^3

4. DICUSSION

In this study, the overall results showed that growth rate, feed conversion ratio and survival were better in treatments II and IV when compared to treatment V and VI. The decline in fish growth rate with increasing stocking densities has been observed in several studies (Vojayan and Leatherland, 1988; Suresh and Lin, 1992; Yousif, 2002, Breine *et al.,* 1996; Aksungur *et al.,* 2007). Besides, it is important to take fish density into account when ranking families or sprogeny groups for growth performance where fish density is an important factor affecting growth and maturation of wild and laboratory fish, besides food supply and its quality, genetics and environmental conditions (Smith *et al.,* 1978). The weekly weight gain indicated that at the lower stocking densities of 158fish/m^3 and 237fish/m^3 there was a higher weight gain. However, growth of fish appears to be better at lower stocking densities as demonstrated in treatment II and IV in which growth was higher throughout. This observation agrees with Yousif (2002) and Breine *et al.* (1996) who reported that increasing the number of fish (density) would adversely affect fish growth. The observed decreased growth rates with increasing stocking density in this study correspond to observation reported by Breine *et al.* (1996). In this study, although the initial fish size was slightly homogeneous for all treatments, the stocking density had an effect on the final size. Aksungur *et al.* (2007) reported that social interactions through competition for food and/or space could negatively affect fish growth. They explained that higher stocking densities lead to increased stress and energy requirements causing a reduction in growth rates and food utilization. Contrary to this result, Osofero *et al.* (2009) reported increased fish production with increased stocking density, attributed to good feed quality and favourable physico-chemical conditions. Youssouf *et al.* (2007) reported that stocking density acts as inhibitory factor for fish growth. Helser and Almeida (1997) suggested that competition for food could also be a possible factor. Yi *et al.* (1996) and Huang and Chiu (1997) also suggested that tilapia is a territorial and aggressive fish so that density effect on growth might be explainable by their competition for territories as well as the permanent stress caused by overcrowding.

The results of specific growth rate (SGR) in this study are higher than those obtained in other studies. For instance, Iluyemi *et al.* (2010) reported SGR of 0.77 to 1.43% in 223 Red Tilapia and Attipoe *et al.* (2009) observed SGR range of 0.43 to 0.53 in earthen pond culture system. Osofero *et al.* (2009) studied the effects of stocking density on growth and survival of *O. niloticus* and found that there was an inverse relationship between survival rate and stocking rate which exceeded 90% in all treatments. The highest survival rate of 98% was recorded in treatment II while the lowest rate of 94.02% was recorded in treatment VI; there was no significant (P=.05) difference in survival rate among treatments I, III, IV and V. The lowest survival rate observed for treatment VI probably resulted from the high mortality rate recorded in that treatment during weeks 2, 8, 24 and especially week 6 of the experimental period. Generally, the survival rates were very high as it ranged from 94.02% to 98% and did not have an inverse relationship with stocking density. Higher survival rate could be attributed to the favorable environmental conditions throughout the experimental period. This is in agreement with the findings of El-Sherif and El-Feky (2009) who indicated that higher (100%) survival rates could be linked to favorable ecological conditions.

The Feed Conversion ratio (FCR) deteriorated with increasing stocking density in this study as was the case of Osofero *et al.* (2009). Although feed intake increased progressively from a low of 510.39g for treatment I to a high of 1034.58g for treatment VI, the feed conversion ratio deteriorated with increasing stocking density. This is probably suggestive of poor acceptance or utilization of diet. However, fish fed MULTI feed (at lower stocking densities of 158 and 237fishes/m^3) had better growth performance in terms of bi-weekly weight gain and final weight gain than fishes fed NIOMR diet even at lower stocking densities. This may be due to the differences in the protein content and quality of MULTI feed and NIOMR diet. Lovell (1989) stated that protein quality of feed can affect the growth performance of fish and that protein quality is affected by amino acid composition and digestibility. Thus, the percentage of protein in a feedstuff determined by chemical analysis is not synonymous with the amount of protein available from feedstuff to the fish. He stated further that the protein in grains and fibrous feed is less digestible to fish than to farm animals.

Protein-energy ratio which gives optimum combination of protein and energy required for fish could also affect growth performance (Lovell, 1989). Apparently fish fed the higher protein feed (NIOMR) had to 'deaminate' much of the protein and use it for energy which may have stressed the fish and reduced their growth rate.

In this study, feed conversion ratio (FCR) ranged from 6.52 to 13.59. These values are higher than FCR reported by other authors. El-Dakar *et al.* (2008) reported FCR range of 0.99 to 1.17 for Florida Red Tilapia fed on fig yam by-product (FYB). The FCRs of 6.52 to 13.59 in this study is very poor because it is higher than recommended FCR of 1.5 for aquaculture (Stickney, 1979). For FCR, the lower the value the better because it tells you the weight of feed (g or kg) used to produce 1g or 1kg of fish flesh. The differences observed in this study could stem from the difference in feed sources and the particular strain of species used. This explanation is in agreement with Guimaraes *et al.* (2008) that efficient utilization of diet may vary even within single specie because of the particular strain of fish used and the environmental factors.

The most rapid growth and highest survival occurred in treatment II. The application of feed remains an important tool because even at 316fish/m^3 and intensive feeding, water quality remained within the acceptable limit. Because concentrations of DO and other water quality variables never fall below the minimum acceptable levels it would have been expected that a higher stocking rate would likely have resulted in greater production and profit. The semi water flow-through system used in this experiment appeared to be a successful way to control nutrient addition, as there were no differences in most chemical and biological variables among the treatments. This was in agreement with Diana *et al.* (1995) who conducted a similar experiment on effects of stocking density and supplemental feeding in Nile tilapia and similar results were reported. However, it was observed in this experiment that feed intake was highest in treatment VI followed by treatment V which were the two treatments with the highest stocking density of 316 fish/m^3 (Table 3). This result disagrees with those reported by Clark *et al.* (1990) and Zonneveld and Fadholi (1991) for red tilapia. The reduced growth rate observed for the fish reared in high stocking density (316fish/m^3) could be attributed to overcrowding. The crude protein of 32% (Multi feed) used in this experimental diets for *O. niloticus* fingerlings (Table 2) fall within the recommended range of 25% -35% crude protein requirement for tilapia species (Santiago and Lovel, 1988) and 30% - 35% recommended by NRC (1981, 1983) and satisfied the nutrient requirement for tilapias (Jauncy, 2000) while 44.8% CP of NIOMR diet is well outside the recommended levels.

The recorded water quality parameter as indicated in Table 5 showed suitable environmental conditions for rearing *O. niloticus* fingelings during the experimental period. Water temperature ranged from 25.49^{o}C to 25.80^{o}C. These values are in the preferred range for optimum growth for Nile tilapia (Xu *et al.,* 2005; El Sherif and El-Feky, 2009). Boyd (2005) reported that tilapia are rather tolerant to low dissolved oxygen and concentration of 3 to 4 mg/L apparently are not extremely harmful to them even with long-term exposure. The dissolved oxygen range of 10.92 – 296 11.04mg/L in this study was higher than the recommended level of 5 to 8mg/L (APHA 1985) for aquaculture system. This finding was in agreement with the report of Siddiqui *et al.* (1991) who reported decrease in dissolved oxygen concentration with decreased rate of water exchange in *O. niloticus* tank culture. pH ranged from slightly acidic (6.97) to neutral (7.0). El-Sherif and El-Feky (2009) recommended a pH range of 7.0 (neutral) to 8 (basic) as optimum for culture of *O. niloticus*. There were no observed apparent effects on the growth of fish because experimental fish accepted feed aggressively at each time of feeding. Total ammonia nitrogen is often a factor that reduces water quality in intensive aquaculture system (Grommen *et al.,* 2002). In this study, the total ammonia concentration was found to be very low in all the treatments which corresponded with the result by Shnel *et al.* (2002) in similar conditions. Frances *et al.* (2000) reported that growth of fish exposed to 0.36mg/L ammonia concentration was depressed. According to them, some of the energy derived from feed consumption may have to be expended on metabolic maintenance rather than growth.

The success of this experiment in treatments fed Multi feed could be attributed to quality of the diets fed. The observed better growth performance in fish fed Multifeed compared to NIOMR fed fish could be attributed to higher level of carbohydrate inclusion in NIOMR feed (Table 2). In this study, there were significant reduction in growth (P=.05) with increasing stocking density irrespective of the diet (NIOMR and Multi feeds) but Multi feed fish performed better with increasing stocking density due to better quality dietary protein in Multi feed. This finding supports Silva *et al.,* (2000) who studied the effect of stocking density on growth of tetra-hybrid red tilapia and found that final body weight gain was significantly higher at lower density while the highest biomass and feed consumption were observed at higher density. The better performance obtained from Multi feed fed fish in this experiment could be majorly attributed to better dietary protein inclusion in Multi feed.

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