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Research Article

Effect of L-Arginine on the Growth of monosex Fingerling Nile Tilapia (*Oreochromis niloticus* L.)

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ABSTRACT

The present experiment was conducted for 5 weeks to investigate the effects of L-arginine on the growth performance of fingerling Nile Tilapia (*Oreochrommi niloticus*). Nile tilapia were stocked in aquarium and fed regularly with different dose of L-arginine and other isoenergetic diets for all the trials. Weekly sampling was carried out to evaluate body weight and length. Fingerlings having average weight and length 0.31 ± 0.01 gm and 2.71 ± 0.01 cm, respectively were fed with T_1 (SD+0.00143% L-arginine of diet+Vit-E), T_2 (SD+0.0143% L-arginine of diet+Vit-E), T_3 (SD+0.143% L-arginine of diet+Vit-E) and T_4 (SD+Vit-E) for 5 weeks. The net weight gains in T_3 (0.88 ±0.03gm) was significantly higher ($p<0.01$) than those of T_1 (0.72 ± 0.02gm), T_2 (0.79 ± 0.02gm) and T4 (0.66 ±0.02gm). The net length gains in T_3 (1.553 ±0.04cm) was significantly ($p<0.01$) higher than that of T_2 (1.480 ±0.02cm), T_1 (1.390 ±0.01cm) and T_4 (1.290 ±0.03cm). The SGR (%/day) in treatment T3 (2.52 ±0.07) was significantly higher ($p<0.01$) than the treatment T1 (2.07 ± 0.04), T2 (2.26 ±0.04) and T4 (1.90 ±0.04). Significant differences (p<0.01) were observed for survival rates among various dietary treatments. The highest survival rate was found in T_3 (86.67%) and the lowest in T_4 (73.33%). The present study indicates that, supplementation of 0.143% L-arginine in feed might be a good growth indicator for mass culture of tilapia.

INTRODUCTION

Tilapia is an African fish broadly cultured in almost 100 countries around the world (Romana-Eguia *et al.*, 2004) and it is prospectively the most important of all aquaculture fish in the 21st century (Fitzsimmons, 2000). In 1970, tilapia was labeled as the 'aquatic chicken' (Maclean, 1984) and has certain favorable characteristics. Tilapia can survive at low dissolved oxygen, euryhaline, has relatively fast growth and efficient food conversion (Asad *et al.*, 2010). This fish did not flourish in aquaculture and proved to be a pest due to its early maturation and breeding habits in the pond. As a result, producer and consumers regarded the fish as nuisance before 1970. During the 1970's a renewed interest in tilapia culture developed in some Asian countries, including Bangladesh with the introduction of Nile tilapia, *O. niloticus*. Now-a-days due to high protein content, big size, fast growth (6–7 months to grow to harvest size) and palatability, tilapia is in the attention of major aquaculture efforts. However, the production of this species is costly because of protein rich feed supplementation and low market price (Hussain et al 2003). Protein formulated feeds in fish is the most expensive component and important nutrient affecting growth performance, feed costs, nitrogen pollution (Gaylord & Barrows 2009) and fish health (Kiron 2012). Balance of dietary essential amino acids is the leading way to increase protein availability. Arginine is one of the limiting amino acids in plant protein source they have been common protein source in tilapia feed. Therefore, determination of L-arginine requirement in feed for all stages of cultured tilapia considered to be important and cost-effective. Arginine is also important for the production of NO and plays significant roles in the modulation of the immune response (Jobgen *et al.*, 2006). In Nile tilapia, the arginine requirement for fry is ranged between 0.015–0.087g (Santiago & Lovell 1988) and for juvenile 6.03 g (Yirong *et al.*, 2015) has been reported. However, L-arginine requirement for fingerling of Nile tilapia has not been reported. Therefore, the present study was designed to determine the optimum dietary arginine requirement for fingerling tilapia by using growth indices

MATERIALS AND METHODS

Experimental design and diet

Fingerling of monosex Nile tilapia were collected from 'BRAC' Fish Hatchery in Moulivibazar district. Four treatments with three replications of each were used for this experiment. Each treatment was designated as T_1 of L-arginine (0.00143% of feed), T_2 (0.0143% of feed), T_3 (0.143% of feed) and T_4 (0.00 % of feed), respectively (Table 1). The length, width and depth of each aquarium were 73.5 cm, 35.50 cm and 38.00 cm. The total water volume used for the 25 fingerlings was 73.06 litter in each aquarium. A detail of feeding schedule is shown in Table 1.

Here, 0.00143 gm, 0.0143 gm and 0.143 gm L-arginine were used in 100 gm supplemental diet for T_1, T_2 and T_3, respectively. There was no L-arginine in the control treatment (T_4). For each treatment other experimental diets were prepared homogenous except L-arginine. The composition of experimental diet and supplemental diet is given in Table 2.

Feed was given at 20% of body weight per day and every 7 days interval the growth was determined, and amount of feed and L-arginine has been modified in following weeks according to the total weight.

Growth trial

Every 7 days interval in the morning (9.00 to 10.00 am), approximately 15 hours after the last feeding randomly selected 5 fishes were counted and weighted from each replication to determine the following parameters:

a. Length gained = Mean final length – Mean initial length
b. Weight gained = Mean final weight – Mean initial weight
c. % of weight gained = $\frac{\text{weight gained}}{\text{Initial weight}} \times 100$
d. % of length gained = $\frac{\text{Length gained}}{\text{Initial length}} \times 100$
e. Survival rate (%) = $\frac{\text{No. of fish caught}}{\text{No. of Fish released}} \times 100$
f. Specific growth rate (SGR, %/day) = [(Ln final weight − Ln initialweight) /duration in days] × 100

Statistical Analysis

All the collected data were statistically analyzed with the help of "MSTAT-C" program. The mean values of all the treatments were calculated and subjected to analysis of variance for each of the characters performed and mean differences among the treatment were done by Duncan's Multiple Range Test (Gomez & Gomez, 1984). Standard (± error) of treatments means were calculated from the residual mean square in the analysis of variance.

RESULTS AND DISCUSSION

Effect of dietary L-arginine levels on body weight

The mean final weight gain of tilapia (*O. niloticus*) were 1.040 ± 0.02 gm, 1.1 ± 0.02 gm, 1.187 ± 0.03 gm and 0.973 ± 0.01 gm in T_1, T_2, T_3 and T_4, respectively (Table 3). The highest final weight found was 1.187 ± 0.03 gm in T_3 and the lowest was 0.973 ± 0.01 gm in T_4. The highest body weight obtained was 1.187 ± 0.03 gm in treatment T_3, which is significantly higher than the other treatment.

Mean weight gain and % weight gain of tilapia (*O. niloticus*) varied significantly among the three treatments. Mean final weight were 0.72 ± 0.02 gm, 0.79 ± 0.02 gm, 0.88 ±0.03 gm and 0.66 ±0.02 gm and % weight gain were 231.9 ±3.28 gm, 258.5 ±3.70 gm, 291.2 ±3.91 gm and 217.5 ±8.58 gm in T_1, T_2, T_3 and T_4, respectively. The highest weight gain and % of weight was found in T_3 which were significantly higher ($p<0.01$) than those of T_4, T_2 and T_1.

The body weight obtained in treatment T_3, T_2 and T_1 were significantly higher than T_4 which is control (Fig.1). This result supported by the previous work done by other scientists with L-arginine. Han *et al.*, (2013) indicated that fish fed with 2.75 g Arg/100 g diet can induce a higher weight gain (848±23.3 gm) than other groups under the experimental circumstances and fish fed with Arg 1.71 g/100 g diet induced a lower weight gain (575±21.7 gm) and higher level of Arg provides better status of the protein utilization. In other words, significant growth improvement of Japanese flounder was obtained by Arg supplementation when fed with the diet contained low fishmeal. Dietary arginine requirements for channel catfish is 4.3% of total protein content (DoF, 2011-12). The significantly lower values were found in fish fed the diet containing low Arg (1.7 g/100 g diet) (Han *et al*. 2013). Alam *et al.*, (2002), reported that lower Arg supplication (1.65 g/100 g diet) in Japanese flounder diet significantly decreased the final body weight, weight gain (WG) and specific growth rate (SGR), meanwhile no significant difference was observed among the higher Arg groups (higher than 2.05 g/100 g diet). Injection of Arg to brown trout *S. trutta* significantly stimulated the insulin-like growth factor-I (IGF-I) in plasma, where the main role of IGF-I is the regulation of development and growth by mediating growth hormone action (Baños *et al.*, 1999). Santiago and Lovell, (1988) studies with Nile tilapia demonstrated that growth depression could occur when fish were fed diets containing an unbalanced dietary arginine level.

Effect of dietary L-arginine levels on body length

The mean final length gains of tilapia (*O. niloticus*) was different among the three treatments. The mean final lengths of tilapia (*O. niloticus*) were 4.100 ±0.02 cm, 4.187 ±0.02 cm, 4.260 ±0.04 cm and 4.000 ±0.02 cm in T_1, T_2, T_3 and T_4, respectively (Table 4 and fig.2). The highest length was found in T_3 (4.260 ±0.04) and the lowest length in T_4 (4.000 ±0.02). Tilapia fry fed with the control diet (Diet 4) without L-arginine revealed the lowest length gain in this experiment. T_3 (4.260 ±0.04) which contained the highest level of L-arginine showed the highest length gain.

It is speculated that, L-arginine that may increase the growth rate of tilapia fry by stimulating the release of growth hormone and other substances in the body. Dietary L-arginine supplementation was found to enhance the immunity in early weaned piglets (Tan *et al.*, 2011). The higher value of pituitary gland (PG) was found in fish fed with high Arg diets, which indicated that high level of Arg provides better status of the protein utilization (Zhao *et al.*, 2012). These results co-relate the present experimental results. L-arginine is converted in the body into a chemical called nitric oxide. Nitric oxide causes blood vessels to open wider for improved blood flow (Bocchi *et al.,* 2000). These results prove that L-arginine, used in this experiment is a growth promoter of tilapia (*O. niloticus*). Arginine did better growth performance on Japanese flounder juvenile (Furuya *et al.,* 2012) suggest that, the arginine treatment affect serum concentrations of nitrogenous and lipid signaling molecules (glycerophosphorylcholine and myo-inositol) and intestinal bacterial metabolites (He *et al.,* 2008), which increase growth hormone responses (Kanaley *et al.,* 2008). All of these results co-relate with the results of present experiment.

Effect of dietary L-arginine levels on body Specific Growth Rate (SGR)

The final specific growth rates (SGR) of tilapia (*O. niloticus*) in different treatments were 2.07 ± 0.04, 2.26 ±0.04, 2.52 ±0.07 and 1.90 ±0.04 in T_1, T_2 T_3 and T_4, respectively (Table 5). The highest SGR recorded was in T_3 (2.52 ±0.07) and the lowest SGR recorded was in T_4 (1.90 ±0.04).

The SGR was significantly higher in the treatment (T_3) which has high percentage of L-arginine. In this experiment the SGR (%/day) in treatment (T_3) was significantly higher than the treatment T_1, T_2 and T_4. This experiment was supported by the previous study where Lue *et al.,* (2004) stated that SGR showed an incremental trend with dietary arginine level and found the highest SGR of 2.38±0.07 in 2.72% arginine on juvenile grouper. Han et al. (2013) also found the highest SGR (3.52±0.00) in 2.75% arginine on Japanese flounder juvenile.

Effect of dietary L-arginine levels on body Survival Rate

The survival rate of tilapia was estimated for each treatment and shown in Table 6. Significant differences for survival rate were observed among various dietary treatments. The survival rate of tilapia (*O. niloticus*) were 77.33%, 81.33%, 86.67% and 73.33 % in treatment T_1, T_2, T_3 and T_4, respectively. The highest survival rate (SR) (86.67%) was observed by the treatment with diet containing 0.143% L-arginine (T_3) and were significantly higher than those of treatments T_1, T_2 and T_4 showed the lowest survival rate.

This result was supported by previous experiment where Cheng *et al.,* (2011) found the highest survival (91.07%) in red drum feed with 1.72% Arg. Luo *et al.,* (2004) also found the highest survival rate (100%) in juvenile grouper feed with 2.10% Arg. In other experiment Han *et al.,* (2013) found the higher survival rate (94.4%) with 2.76 gm Arg/100 gm diet in Japanese flounder. These results correlate with the results of present study. Supplemental arginines (Arg) improved the immunologic response and reduce mortality in rodents with sepsis (Luo *et al.,* 2004). Bocchi et al. (2000) reported that L-arginine might be used to build the body or immune-system of the body and considered as 100% digestible. Fish fed increasing dietary lysine showed quadratic effect on the protein deposition ratio (Furuya at al., 2012). This results also support that why the survival rate of tilapia treated with L-arginine was high than that of without L-arginine.

CONCLUSION

In recapitulation, the present study confirms the action of arginine and indicates that a gradual increase in the dose of supplemental arginine improved the growth performance of tilapia in *O. niloticus*. Supplementation with L-arginine enhanced the feed efficiency of tilapia as well as promotes the development of the intestine. The present study revealed that, L-arginine played a significant role in increasing the growth of fingerling tilapia. This also helped to increase in survival rate of the experimental fish. In respect of tilapia weight, length, SGR and survival rate, T_3 treatment (0.143% L-arginine of diet) performed the best growth performance. Considering weight, the tilapia fingerling, that were cultured without L-arginine (T_4) performed lowest compared to others. The maximum survival rate (86.67%) showed by the tilapia cultured with 0.143 gm L-arg/100gm diet while it showed the SR of 73.33% only when cultured without L-arginine. Finally, it can be stated that incorporation of L-arginine could increase the production far better.

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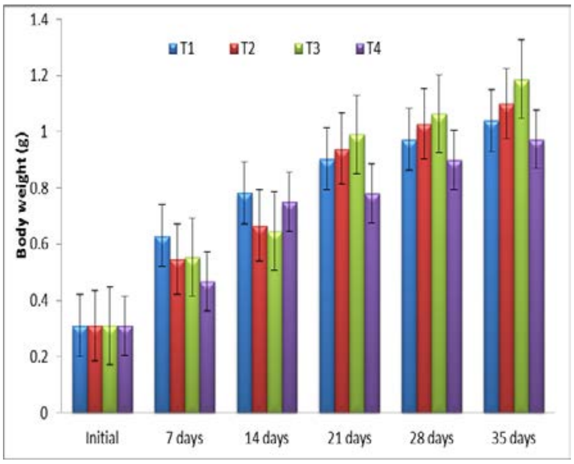

Fig. 1: Variation of body weight (g) during 35 days' experiment

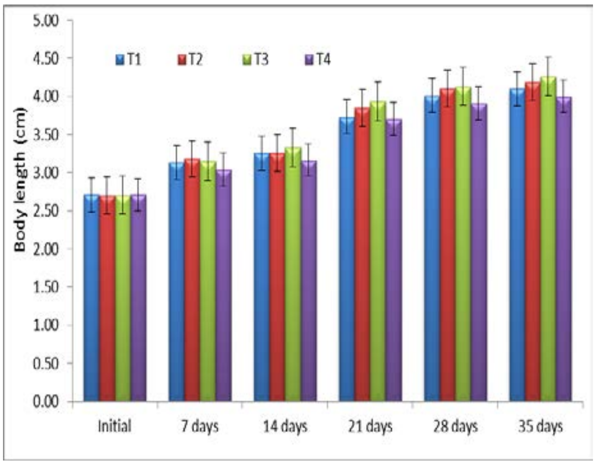

Fig. 2: Variation of body length during 35 days' experiment

Table 1: Layout of the experiment for growth performance of *o. niloticus*

Treatment	L-arginine (% of feed)	Feed	Replication	Size of aquarium(m^3)	Water volume (litter)	Stocking density
T_1	0.00143	*SD	R1	0.992	992	25
			R2	0.992	992	25
			R3	0.992	992	25
T_2	0.0143	SD	R1	0.992	992	25
			R2	0.992	992	25
			R3	0.992	992	25
T_3	0.143	SD	R1	0.992	992	25
			R2	0.992	992	25
			R3	0.992	922	25
T_4	0	SD	R1	0.992	992	25
			R2	0.992	992	25
			R3	0.992	992	25

*SD= Supplemental Diet

Table 2: Ingredient of feed used in experiment and proximate composition of the supplemental diet (SD)

Treatment	Feed ingredient	Proximate composition of the supplemental diet (SD)
T_1	20% SD of fish body weight + 0.00143% L-arginine of feed wt.	Rice bran 21%, Wheat bran 15%, Wheat flour 10%, Fish meal 40%, Maize meal 13%, Vit-B complex 0.5%, Vit-E 0.5%
T_2	20% SD of fish body weight + 0.0143% L-arginine of feed wt.	
T_3	20% SD of fish body weight + 0.143% L-arginine of feed wt.	

Table 3: Weight of Tilapia (O. *niloticus*) in aquarium during the experiment period in four different treatments

Treatment	**Body weight (gm)**					
	Initial (M ± SE)	**7 days** (M ± SE)	**14 days** (M ± SE)	**21 days** (M ± SE)	**28 days** (M ± SE)	**35 days** (M ± SE)
T_1	0.31±0.01	0.6300 ±0.03^a	0.7833 ±0.02^a	0.9033±0.03^b	0.973±0.01^c	1.040±0.02 ^{c}
T_2	0.31±0.01	0.5467 ±0.02^b	0.6667±0.01^c	0.9400±0.00^{ab}	1.027±0.01^b	1.100±0.02 ^{b}
T_3	0.31±0.01	0.5533 ±0.02^b	0.6467±0.01^d	0.9900±0.05 ^{a}	1.063 ±0.02^a	1.187±0.03 ^{a}
T_4	0.31±0.01	0.4667 ±0.01^c	0.7500±0.01^b	0.7800±0.04^c	0.900±0.02 ^{d}	0.973±0.01^d
CV%	1.88±0.01	4.07	1.72	3.64	1.51	2.01
Level of significance	NS	**	**	**	**	**

In a column, means followed by common letters are not significantly different from each other at 5% level of probability by DMRT. **= Significant at (p<0.01), *= Significant at (p<0.05), NS= Non-significant

Table 4: Length of Tilapia (*O. niloticus*) in aquarium during the experimental period in four different treatments

Treatment	**Length (cm)**					
	Initial (M ± SE)	**7 days** (M ± SE)	**14 days** (M ± SE)	**21 days** (M ± SE)	**28 days** (M ± SE)	**35 days** (M ± SE)
T_1	2.71±0.01	3.133±0.04^a	3.253 ±0.05^a	3.733 ±0.02^c	4.013±0.02^c	4.100 ±0.02^c
T_2	2.71±0.01	3.180±0.03^a	3.260±0.03^a	3.853 ±0.05^b	4.107±0.01^b	4.187 ±0.02^b
T_3	2.71±0.01	3.153 ±0.03^a	3.327±0.01^a	3.933±0.013^a	4.133±0.01^a	4.260 ±0.04^a
T_4	2.71±0.01	3.040 ±0.05^b	3.167 ±0.06^b	3.707 ±0.01^c	3.913±0.03^d	4.000 ±0.02^d
CV%	0.35	1.31	1.30	0.84	0.52	0.65
Level of significance	NS	*	*	**	**	**

In a column, means followed by common letters are not significantly different from each other at 5% level of probability by DMRT. **= Significant at (p<0.01), *= Significant at (p<0.05), NS= Non-significant

Table 5: ***Specific Growth Rate*** of Nile tilapia (*o. niloticus*) in aquarium after 35 days experimentation

Treatment	**Parameters**				
	Weight gain (g) (M ± SE)	Length gain (cm) (M ± SE)	%Weight gain (g) (M ± SE)	% Length gain (cm) (M ± SE)	SGR (M ± SE)
T_1	0.72 ± 0.02[c]	1.390 ±0.01[c]	231.9 ±3.28[c]	51.29 ±0.18[c]	2.07 ± 0.04[c]
T_2	0.79 ± 0.02[b]	1.480 ±0.02[b]	258.5 ±3.70[b]	54.68 ±0.78[b]	2.26 ±0.04[b]
T_3	0.88 ±0.03[a]	1.553 ±0.04[a]	291.2 ±3.91[a]	57.38 ±1.20[a]	2.52 ±0.07[a]
T_4	0.66 ±0.02[d]	1.290 ±0.03[d]	217.5 ±8.58[d]	47.60 ±1.12[d]	1.90 ±0.04[d]
CV%	2.28	1.73	2.13	1.73	2.28
Level of significance	**	**	**	**	**

In a column, means followed by common letters are not significantly different from each other at 5% level of probability by DMRT. **= Significant at (p<0.01), *= Significant at (p<0.05), NS= Nonsignificant

Table 6: Survival rate (*O. niloticus*) in aquarium after 35 days experimentation

Treatment	**Survival rate%** (M ± SE)
T_1	77.33 ± 2.31[bc]
T_2	81.33 ± 2.31[b]
T_3	86.67 ± 2.31[a]
T_4	73.33 ± 2.31[c]
CV%	2.90
Level of significance	**

In a column, means followed by common letters are not significantly different from each other at 5% level of probability by DMRT. **= Significant at (p<0.01), *= Significant at (p<0.05). NS= Non-significant