# Effect of L-Arginine on the Growth of Tilapia (*Oreochromis niloticus*)

A Thesis

By

Mariya Akter

Examination Roll No: 1202030506, Reg. No. 0561

Session: 2007-2008, Semester: July-December, 2013

Submitted to the
Department of Fish Biology and Genetics
Faculty of Fisheries
Sylhet Agricultural University, Sylhet-3100
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

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Approved as to the style and contents by

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# ABSTRACT

Arginine plays important roles in tissue repair, cell replication and collagen synthesis which impact animal growth and survival. An experiment was carried out in triplicate to evaluate the effects of L-arginine on the growth of Tilapia (*O. niloticus*) in twelve aquaria in "Fish Biology and Genetics Laboratory", under the Faculty of Fisheries, Sylhet Agricultural University, during 23rd July to 28th August 2013. Tilapia of 45 days were stocked in glass aquarium and fed regularly with different dose of L-arginine. Sampling was done weekly and recorded properly to evaluate improvement of body weight and length. *O. niloticus* fries 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 gain of fish 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 gain of fish in $T_3$ (1.553 ±0.04cm) which 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). Survival rate of fish were 77.33, 81.33, 86.67 and 73.33% in $T_1$, $T_2$, $T_3$ and $T_4$, respectively. However total net profit/treatment/35days of $T_3$ (Tk. 37.93) was significantly higher (($p$<0.01) than those of $T_2$ (Tk. 32.24), $T_1$ (Tk. 26.04) and $T_4$ (Tk. 15.54). Benefit cost ratio (BCR) $T_3$ (1.76) was higher than those of $T_2$ (1.75), $T_1$ (1.61) and $T_4$ (1.38). Measured water quality parameters in different treatments during the experimental period were found to be similar and ranges were within acceptable limit for tilapia culture. On the basis of observed results, we can conclude that supplemental feed with 0.143% L-arginine might be a good growth indicator and profitable for mass culture of tilapia (*O. niloticus*).

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**THE AUTHOR**

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## List Of Abbreviation

| Abbreviation | Full Name |
|---|---|
| AIT | Asian Institute of Technology |
| Arg | Arginine |
| BBS | Bangladesh Bureau of Statistics |
| BFRI | Bangladesh Fisheries Research Institute |
| BRAC | Bangladesh Rural Advancement Committee |
| DBP | Diastolic Blood Pressure |
| DEGITA | Dissemination and Evaluation of Genetically Improved Tilapia in Asia |
| DMRT | Duncan's Multiple Range Test |
| DO | Dissolved Oxygen |
| DoF | Department of Fisheries |
| EAA | Essential Amino Acid |
| ED | Erectile Dysfunction |
| FAO | Food and Agricultural Organization |
| FCR | Feed Conversion Ratio |
| FE | Feed Efficiency |
| g/kg | Gram / Kilogram |
| GDP | Gross Domestric Production |
| GIFT | Genetically Improved Farmed Tilapia |
| HPSV | Hydropericardium Syndrome Virus |
| Ig | Immunoglobulin |

| | |
|---|---|
| IGF | Insulin-Like Growth Factor |
| IL | Interleukin |
| MAP | Mean Arterial Blood Pressure |
| MT | Metric Ton |
| NGO | Non Government Organization |
| NO | Itric Oxide |
| NO | Nitric Oxide |
| NOx | Nitrates/ Nitrites |
| PAD | Peripheral Arterial Disease |
| PER | Protein Efficiency Ratio |
| PG | Pituitary Gland |
| PR | Protein Retention |
| SBP | Systolic Blood Pressure |
| SGR | Specific Growth Rate |
| SR | Survival Rate |
| Tr | Treatment |
| UNICEF | United Nations Children's Emergency Fund |
| WG | Weight Gain |

# CHAPTER ONE

## INTRODUCTION

Bangladesh is a densely populated country of 147570 $km^2$ with a population of 149.8 million people (BBS, 2011). Fisheries sector contributed 4.39% to national GDP and 22.76% to the agricultural GDP and 2.46% to foreign exchange earnings by exporting fish products in 2012 (DoF, 2012). Fish provides 60% of national animal protein consumption. Fisheries sector also plays an important role in rural employment generation and poverty alleviation. Bangladesh is one of the world's leading inland fisheries producers. Inland fisheries comprises of rivers, ponds, estuaries, beels, floodplains, haors, baors, brackish water etc. There are 260 fish and 24 prawn species in inland fresh water in the country. In early sixties inland fisheries contributed about 90% of total fish production of the country. Fish production from aquaculture has increased to a great extent but open water fish production is in slow progress. Now only about 34% of total fish production comes from inland open water (DoF, 2010-2011).

In 2011-12 the total fish production is 32.62 lakh Metric Ton. Inland open, inland closed and marine water bodies represent 957095, 1726067 and 578620 MT, respectively. Average annual growth rate of fish production in last 3 years is 6.11%. The Production from closed water bodies is increasing very sharply due to dissemination of adaptive technologies and need-based extension services rendered by DoF.

Indian major carps and exotic carps are largely cultured in the country. Among exotic carps, tilapia is one of the promising species which is culturing throughout the country. It should be mentioned here that tilapia has brought earlier in this country. During that time, this species was unpopular in the culture system. Popularity of this species to the farmers is negligible due to lack of knowledge of farmers on biology and culture of tilapia. Tilapia contributes in fish production with expansion of culture and innovation of new technology.

Therefore, a significant effort to increase fish production should be concentrated on aquaculture. Bangladesh is brought face to face today with the problem of protein calorie malnutrition. To overcome these problems of protein malnutrition increase of fish production is essential. Tilapia is one of the faster growing and important species of culture fishery and low capital investment but with promise of high financial returns.

Tilapia has become the second most important cultured fish group in the world after carps. Tilapia culture is also one of the fastest growing farming activities, with an average annual growth rate of 13.4% during 1970–2002. They are widely cultured in about 100 countries in the tropical and subtropical regions. As a result, the production of farmed tilapia has increased from 2.5 million in 2007 to 3.6 million in 2008. (FAO, 2010). Tilapia is called the aquatic chicken and found everywhere in Bangladesh.

Among tilapias, *Oreochromis mossambicus* was the first species, which was introduced into Bangladesh from Thailand in 1954. The fish did not flourish and proved to be a pest due to its early maturation and profile breeding habits in the pond. As a result, producer and consumers regarded the fish as nuisance fish. 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*, it is known as aquatic chicken. In 1974, the chitralada strain of nile tilapia, a promising farmed species, was introduced into Bangladesh from Thailand through UNICEF. The Bangladesh Fisheries Research Institute (BFRI) initiated the second introduction of the fish in this country, also from Thailand in 1987. Meanwhile, a red mutant tilapia, a hybrid between albino *O. mossambicus* x *O. niloticus*, was developed in Taiwan and introduced into Thailand. In 1988 Drs. M. G. Hussain and S. Dewan brought a batch of this red strain of tilapia to Bangladesh from the Asian Institute of Technology (AIT), Bangkok, Thailand. Under the Dissemination and Evaluation of Genetically Improved Tilapia in Asia (DEGITA) project of World Fish Center, another promising Genetically Improved Farmed Tilapia (GIFT) strain, a synthetic strain of *O. niloticus*, was introduced in July 1994 from the Philippines.

Protein is the most expensive component in fish feed and also the most important nutrient in growth performance. The selection of proper quantity and quality of dietary protein is a necessary tool for successful tilapia culture practices. However, information of the gross protein requirement is of limited value without data on the essential amino acid (EAA) requirement because protein quality depends on its amino acid composition and on the digestibility. Arginine is an EAA required by all fish species investigated to date (LUO *et al*. 2004).

**Arginine** (**Arg**) is an α-amino acid. The L-form arginine (L-Arginine) is one of the 20 most common natural amino acids. Preterm infants are unable to synthesize or create arginine

internally, making the amino acid nutritionally essential for them. There are some conditions that put an increased demand on the body for the synthesis of L-arginine, including surgical or other trauma, sepsis and burns. Arginine was first isolated from a lupin seedling extract in 1886 by the Swiss chemist Ernst Schultze. In 1932, scientists learned that L-arginine is needed to create urea, a waste product that is necessary for toxic ammonia to be removed from the body. In 1939, researchers discovered that L-arginine is also needed to make creatine. Creatine breaks down into creatinine at a constant rate, and it is cleared from the body by the kidneys.

L-arginine is a chemical building block called "an amino acid." It is obtained from the diet and is necessary for the body to make proteins. It can also be made in a laboratory and used as medicine. Individuals who have poor nutrition or certain physical conditions may be advised to increase their intake of foods containing arginine.

Arginine is one of the fastest growing factors of the global livestock production (FAO, 2012). Arginine plays an important role in cell division, the healing of wounds, removing ammonia from the body, immune function, and the release of hormones. L-arginine is also used for heart and blood vessel conditions, recurrent pain and erectile dysfunction and preventing common cold, improving kidney function, control blood pressure and perform many other functions in human body. The benefits and functions attributed to oral supplementation of L-arginine include:

- Precursor for the synthesis of nitric oxide (NO)
- Reduces healing time of injuries (particularly bone)
- Quickens repair time of damaged tissue
- Helps decrease blood pressure in clinical hypertensive subjects.

Although no experiment of such kind has yet been conducted on fish to increase its growth and survival rate. Very little work has been done on fish. Some information and studies are available on pig, rat and mammals. Therefore the experiment was designed to know the effect of L-arginine on growth of tilapia.

Considering the above facts, studies conducted with the following objectives:

- To study the effects of L-arginine in the growth of tilapia.
- To recommend L-arginine in tilapia feed for aquaculture.
- To observe the economic benefit of L-arginine on tilapia.

# CHAPTER TWO
# REVIEW OF LITERATURE

Growth performance of tilapia (*Oreochromis niloticus*) by L-arginine is a new chapter in aquaculture. Information regarding the experiment is not available. There is no such work has been done on fish by L-arginine but work has been done on other animals like pig, rats, human etc. 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. L-arginine also stimulates the release of growth hormone, insulin, and other substances in the body. Arg was reported to be one of the most versatile amino acids (Li et al., 2009), which was also reported to present a unique role in treating many developmental and health problem among amino acids (Wu, 2009). L-arginine as biofunctional compounds is an effective way to enhance growth performances of tilapia. An attempt has been made to present a short review of available literature to the present studies.

Han *et al*. (2013) conduct a study on Japanese flounder juvenile and reported that arginine and histidine did better growth performance. Better nutrient utilizations (feed conversion ratio, protein efficiency ratio and protein retention) were also observed in higher Arg supplemented groups. There were no significant effects on the hematocrit, hemoglobin, glucose, total cholesterol, total bilirubin and mucus bactericidal activity of experimental fish among treatments.

Furuya *et al.* (2012) conducted a study to determine the digestible lysine requirements of nile tilapia fingerlings. With increasing levels of lysine in the diet, a quadratic effect on weight gain, feed conversion, protein efficiency ratio, protein deposition rate, deposition rate of fat, body moisture and body lipids was observed, where the best values of the variables were estimated at 15.96, 16.4, 14.35, 15.21, 15.87, 15.21 and 16.29 g/kg of lysine, respectively. The digestible lysine requirement of Nile tilapia fingerlings is 15.21 g/kg (5.41 g/100 g of digestible protein), in diets balanced for the arginine:lysine ratio.

Tan *et al*. (2011) reported that dietary L-arginine (Arg) supplementation promotes muscle gain and reduces body-fat accretion of pig. Serum concentrations of leptin, alanine and glutamine were lower, but those for arginine and proline were higher in Arg-supplemented pigs than in control pigs. The percentage of oleic acid was higher but that of stearic acid and linoleic acid was lower in muscle of Arg-supplemented pigs, compared with control pigs.

Cheng *et al*. (2011) noted feeding trial ranged from 89 to 92% of L-arginin for *Sciaenops ocellatus* shows no significant differences were observed among treatments. Enhanced weight gain and feed efficiency were generally observed in red drum fed the diets supplemented with arginine and/or glutamine compared to those fed the basal diet, although statistical differences were not consistently achieved. However, fish fed the diets supplemented with glutamine at 2% or arginine and glutamine both at 1% did have significantly (Pb0.05) higher feed efficiency.

Cheng *et al.* (2011) reported that feed efficiency of fish was significantly (Pb0.05) improved by supplementation of glutamine at 2% and the combination of both arginine and glutamine at 1% of diet. Neutrophil oxidative radical production in fish fed the glutamine and/or arginine-supplemented diets was significantly (P=0.03) higher compared with that of fish fed the basal diet, with a synergistic effect observed in fish fed the combined arginine-glutamine diet.

Tan B *et al*. (2009) studied dietary L-arginine supplementation enhances immunity in early weaned piglets. Seventy piglets weaned at 7 days of age were assigned to five groups (14 pigs/group), representing supplementation of 0.0, 0.2, 0.4, 0.6, and 0.8% L-arginine to a milk-based formula. On day 7 after initiation of treatment, spleen weight in piglets supplemented with 0.2 and 0.8% arginine was heavier and thymus size was larger in piglets supplemented with 0.6% arginine. Dietary supplementation with 0.8% arginine increased the numbers of white blood cells and granulocytes, and gene expression of interleukin (IL)-8 in spleen. On day 14, compared with control piglets, granulocyte numbers were greater but lymphocyte numbers were lower in piglets supplemented with 0.2 and 0.4% arginine, whereas splenic expression of IL-8 and tumor necrosis factor-alpha genes was increased in piglets supplemented with 0.8% arginine. Additionally, IgG and IgM concentrations in serum and growth performance were greater in piglets supplemented with 0.4-0.8% arginine, compared with unsupplemented piglets.

He Q *et al*. (2009) conduct a study to determine the effect of dietary arginine supplementation on the metabolome in serum of growing pigs using (1) H nuclear magnetic resonance spectroscopy. Arginine played an important role regulating nutrient metabolism, but the

underlying mechanisms were largely unknown. The arginine treatment affected serum concentrations of nitrogenous and lipid signaling molecules (glycerophosphorylcholine and myo-inositol) and intestinal bacterial metabolites (formate, ethanol, methylamine, dimethylamine, acetate, and propionate). These novel findings suggest that dietary arginine supplementation alters the catabolism of fat and amino acids in the whole body, enhances protein synthesis in skeletal muscle, and modulates intestinal microbial metabolism in growing pigs.

K. Munir *et al*. (2009) conducted a research on the effects of dietary supplement of arginine on protective humoral and cell-mediated immune responses of broiler chicks vaccinated and challenged against hydro pericardium syndrome virus (HPSV) and found that the dietary supplementation of arginine had beneficial effects on humoral and cell-mediated immune responses of broiler chicks against HPSV.

JA Kanaley (2008) found that the resting growth hormone responses increase with oral ingestion of L-arginine and the dose range is 5-9 g of arginine. Within this range there is a dose-dependent increase and higher doses are not well tolerated. L-arginine ingestion will enhance the growth hormone response and the combination of arginine plus exercise increases growth hormone, but this increase may be less than seen with exercise alone. This diminished response is seen in both in both younger and older individuals.

Z. Zhan *et al*. (2008) evaluated that the effects of dietary arginine levels on micro vascular development of the small intestine in early-weaned pigs. Twenty-four crossbred pigs (5.0 +/- 0.3 kg body weight) were individually housed and randomly allotted to 1 of 3 diets supplemented with 0, 0.7, and 1.2% L-arginine (8 pigs per group). Pigs consumed the diets and libitum for 10 d. The blood samples were collected on d 3, 6, and 10. On d 10, 6 pigs from each group were randomly selected and killed for tissue sample collection. Compared with control pigs, dietary supplementation with 0.7% L-arginine increased ($P < 0.05$) jejuna concentrations of nitrite and nitrate (stable oxidation products of nitric oxide), intestinal villous height, as well as plasma proline and arginine concentrations on d 6 and 10.

A. M. Wilson *et al.* (2007) found that patients with PAD, long-term administration of L-arginine does not increase nitric oxide synthesis or improve vascular reactivity. Placebo effect observed in studies of functional capacity was attenuated in the L-arginine-treated

group. The short-term administration, long-term administration of L-arginine is not useful in patients with intermittent claudication and PAD.

K. Rytlewski *et al*. (2005) conducted a study on the effect of L- arginine supplementation and investigated that, the influence of dietary supplementation with l-arginine on blood pressure and biochemical measures of NO production in women with preeclampsia in prospective, randomized, placebo-controlled study, treatment with exogenous l-arginine significantly elevated 24-h urinary excretion of NOx and mean plasma levels of l-citrulline. Exogenous l-arginine did not influence plasma concentrations of l-arginine, l-ornithine and methylated arginines. Prolonged dietary supplementation with l-arginine significantly decreased blood pressure through increased endothelial synthesis and/or bioavailability of NO.

Suarez Butler MF *et al*. (2005) conducted a research on arginine supplementation to develop antigen specific immunity in mice. Arginine is a conditionally essential amino acid with many physiologic roles. Its role in immune function has been one of major focus with conflicting results. The data resulted from the research work suggest that despite significant enhancement of in vitro mitogen-induced splenocyte proliferation, arginine supplementation does not have a biologically significant effect on antigen-specific in vivo indicators of immune function in this model.

J. C. Moriguti *et al*. (2005) conducted a study on the effect of arginine supplementation on the humoral and innate immune response of older people. This study suggests that after the pneumococcal vaccine, the intake of arginine increased neutrophil chemotaxis, natural killer cytotoxicity and serum concentration of IgG against antigen 5 in older people. These results suggested that arginine supplementation might enhance the immune response elicited by the pneumococcal vaccine in older people.

SW. Kim and G. Wu. (2004) conducted a research programmed to determine the effect of dietary arginine supplementation on the growth of artificially reared piglets. The pigs (n = 24; 7 d old) were removed from sows to a nursery facility and assigned randomly to 1 of the 3 treatments representing diets supplemented with 0, 0.2, or 0.4% L-arginine (on the basis of milk replacer powder). Each milk feeder was assigned to 1 dietary treatment. Body weights of piglets were measured and jugular venous blood samples were obtained for metabolite analysis at d 7, 14, and 21 of age. Compared with control piglets, dietary supplementation with 0.2 and 0.4% L-arginine dose dependently increased ($P < 0.05$) plasma concentrations of arginine by 30 and 61%, and decreased ($P < 0.05$) plasma concentrations of ammonia by 20

and 35%, and those of urea by 19 and 33%, respectively. Both the metabolic and growth data demonstrate unequivocally that arginine is deficient in milk-fed young pigs and that this arginine deficiency represents a major obstacle to maximal growth in piglets.

HF. Shang *et al*. (2004) found that supplemental arginine (Arg) improved the immunologic response and reduce mortality in rodents with sepsis. Rats were assigned to four groups. Groups 1 and 2 were fed a semi purified diet, while in the diets of groups 3 and 4, part of the casein was replaced with Arg. Total lymphocyte yields in Peyer's patches, and small intestinal immunoglobulin A (IgA) secretion in group 4 were significantly higher than the groups 1 and 2. No differences were observed between groups 3 and 4. There were no differences in the interleukin (IL)-2 and interferon- gamma levels among all groups when splenocytes were stimulated with mitogen.

I. Miguez *et al*. (2004) conducted a study to investigate the effect of dietary L-arginine supplementation on serum lipids and intestinal enzyme activities in diabetic rats. Control and diabetic rats were fed diets with or without 2% L-arginine supplementation for 4 weeks. Diabetic rats had significantly higher concentrations of serum triglycerides and LDL-cholesterol than control rats. These alterations were partially reduced by L-arginine supplementation.

RB. Preli *et al*. (2002) said that, many investigators have been interested in whether dietary L-arginine supplementation can augment nitric oxide production and thereby improve vascular health. The effects of oral L-arginine on vascular health and disease have been examined both in human beings and in various animal models. The results of studies of oral L-arginine supplementation on atherosclerotic lesion formation, as well as markers of endothelial function (e.g. macrophage function, platelet aggregation and adhesion, and in vitro vascular ring studies). Although results of oral L-arginine supplementation in hypercholesterolemic animals have generally shown beneficial effects, the data in humans are varied, possibly because of small sample sizes and brief periods of study. Long-term randomized clinical trials are needed to more definitively address whether oral L-arginine supplementation could be advantageous for vascular health.

Fan *et al.* (2001) found that interaction between lysine and arginine may take place during intestinal absorption in poultry, in which plasma levels of these two amino acids do not

reflect dietary concentrations. In contrast, decreased plasma arginine concentrations were not observed in dogs and rats fed excess lysine. In the present study, fish fed excess lysine levels diets in adequate in arginine had lower plasma arginine concentrations when compared to the control group. The surplus arginine and lysine supplemented in diet seemed to have enhanced hepatic arginase activity. The lowest arginase activity was observed in the livers of black sea bream fed the highest dietary lysine levels.

Buentello and Gatlin (2000) found in a research weight gain (WG) and plasma amino acid concentrations of fish fed diets with glycine suggested that it does not serve as a precursor for citrulline. Based on WG and feed efficiency (FE), juvenile channel catfish were found to require arginine at 3.3% to 3.8% of dietary protein, when glutamate was included in the diet. The requirement estimate was 33% higher when glycine replaced glutamate in the diet and was similar to the previously determined arginine requirement of channel catfish at 4.3 gr100 g of dietary protein. These results strongly suggest that dietary glutamate is used for endogenous synthesis of arginine in channel catfish, especially when arginine is deficient in the diet.

Novaes *et al.* (2000) reported that the effects of diet arginine supplementation for rats with Walker 256 solid tumor are controversial. Intragastrical solutions with arginine at 4% and 6%, a standard diet (control) were administered to the animals. Surviving time of the rats with solid tumor did not vary significantly between the groups. The rate of metastase was lower in animals with Walker 256 solid tumor supplemented with arginine. The amino acid metabolism was estimulate in the animals after arginine supplementation at 4% and 6%, demonstrated by significant increases in blood levels of arginine, ornitine, citruline, proline and histidine when compared to the control group. Anaemia was less severe in the rats with Walker 256 solid tumor that received arginine supplementation. The results suggest that arginine 6% supplementation may have pharmacologic effect in rats with Walker 256 solid beyond the nutritional one.

# CHAPTER THREE
# MATERIALS AND METHODS

## 3.1 Study Area

The experiment was conducted in post-graduate laboratory of Fish Biology and Genetics Department, Faculty of Fisheries, Sylhet Agricultural University. The University campus with an area of 50 acres is located in scenic rural surrounding on the 6 km east of Sylhet. The experiment was undertaken using rectangular shaped twelve aquaria.


**Figure 3.1: Location of the Study Area**

## 3.2 Sample Collection

Forty five days old fries of monosex tilapia were collected from 'BRAC' Fish Hatchery in Moulivibazar district and kept into an oxygenated polybag. The polybag used in fries transportation was of 74 cm x 46 cm dimension and 0.062 mm thickness. At first the bag was put into a tin or any rigid box of 18-20 litter capacity and the bag was filled up to 1/3 of its capacity (6-7 liters) with water and then 1000 fries were put into it and finally it was inflated

with oxygen in high pressure from a cylinder, up to two third of the bag. The upper 10-15 cm of the bag was twisted, bends and tied securely air tight with a string. Then the bags were carried out into the university lab by a microbus.

**Figure 3.2: Transportation of Live Tilapia Fry in Oxygenated Poly Bag**

### 3.3 Design of Experiment

Four treatments were used for this experiment. Each treatment had three replications. The design of experiment is shown in Table 3.1. Each treatment had designated as $T_1$, $T_2$, $T_3$ and $T_4$. $T_1$ 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. The length, width and depth of each aquarium were 73.5 cm, 35.50 cm and 38.00 cm, respectively. The total water volume used for the 25 fries was 73.06 litter and individual fingerlings used 2.92 liter water in each aquarium. A detail of feeding schedule is shown in Table 3.2.

**Table 3.1: 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/treatment |
|---|---|---|---|---|---|---|
| $T_1$ | 0.00143 | *SD | R1<br>R2<br>R3 | 0.992 | 992 | 25<br>25<br>25 |
| $T_2$ | 0.0143 | SD | R1<br>R2<br>R3 | 0.992 | 992 | 25<br>25<br>25 |
| $T_3$ | 0.143 | SD | R1<br>R2<br>R3 | 0.992 | 992 | 25<br>25<br>25 |
| $T_4$ | - | SD | R1<br>R2<br>R3 | 0.992 | 992 | 25<br>25<br>25 |

*SD= Supplemental Diet

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 ($T_4$). Feed were given in each replication under the treatment as the same as each treatment.

**Table 3.2: The Ingredient of Feed Used in Experiment**

| Treatment | Feed ingredient |
|---|---|
| $T_1$ | 20% SD of fish body weight + 0.00143% L-arginine of feed wt. |
| $T_2$ | 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. |
| $T_4$ | 20% SD of fish body wt. |

**Figure 3.3: Layout of Experimental Unit**

### 3.4 Water Supply

Tap water from university central supply system was used in this experiment. The water was allowed to hold in a reservoir for 6 hrs as to settle down any suspended particles and to remove elemental odors. The water quality parameters were monitored before use them in the aquaria.

### 3.5 Physico-Chemical Parameter

Physico-chemical parameters like temperature, pH and dissolved oxygen of the water of aquarium were measured twice per week.

Temperature and dissolved oxygen were measured by a digital DO meter (YSI model 58) and pH with a pH electrode (Jenway, model 3020). Before taking a measurement pH meter was properly adjusted with buffer solution pH-7. Recording of water temperature, dissolved oxygen, pH was done between 08.00 -09.00 am. Other parameters were monitored by HACH kit.

### 3.6 Diet Preparation

Supplemental Diet (SD) was prepared according to the Table 3.3.

**Table 3.3: Proximate Composition of the Supplemental Diet (SD)**

| Components | Percentage |
|---|---|
| Rice bran | 21% |
| Wheat bran | 15% |
| Wheat flour | 10% |
| Fish meal | 40% |
| Maize meal | 13% |
| Vit -B complex | 0.5% |
| Vit - E | 0.5% |

Feed was given at 20% of body weight per day. Every 7 days interval the growth is determine and change the amount of feed and L-arginine according to the total weight.


**Figure 3.4: L-arginine Used in this Experiment**

### 3.7 Experimental Procedure

The experiment was conducted from 23rd July 2013 to 28th August 2013. Healthy and uniformed sized fries was collected from a commercial hatchery with initial mean weight of 0.31 gm and initial mean length 2.71 cm and kept in 15 litter rectangular aquariums for 2 hrs acclimatization. Fry were stocked in aquarium according to the design of the experiment.

**Figure 3.5: Feeding of Tilapia Fry in the Aquarium (left); Filtration System in the Aquarium (right)**

**Figure 3.6: Rearing Tilapia Fry in the Aquarium**

Two gm L-arginine normally being used for 70 kg human body weight. There is no such work of L-arginine on fish growth. So the dose of L-arginine used in this experiment was based on human dose. Therefore, 0.00022 gm L-arginine for 25 fries (total body weight 7.75 gm) in an aquarium was used.

The aquarium was provided with sand and iron filtered, freshwater (2 litter/min) and with continuous aeration to maintain the dissolved oxygen level near saturation. Tilapia were fed twice daily at 09.00 am and 4.00 pm. Fish were weighted in bulk every weeks and measured the length until the termination of the experiment (5 week). Fecal matter was removed by siphoning at each morning before morning feeding and at each afternoon before afternoon feeding and half of the water replaced with the filtered water twice daily after the removal of the fecal matter and before the feeding. Mortality was cheeked daily.

The experiment was conducted at ambient temperature with a natural photoperiod (approximately 12 hrs light/ 12 hrs dark). Water temperature and salinity were monitored daily between 8 to 9 am, while pH, ammonia nitrogen and dissolved oxygen were monitored twice weekly.

Every 7 days interval in the morning (9.00 to 10.00 am), approximately 15 hrs after the last feeding all fish were counted and randomly selected 5 fishes were weighted from each replication to determine weight gain, specific growth rate and length increase. Weight was taken with a spring balance (DONGIL-15 kg x 50 g) and length with a measuring scale. All

the data recorded in a note book and finally calculated the average length and weight of fishes according to treatment on each sampling day. After taking the data, all the fishes were kept to their own aquarium carefully.

At the termination of the 5 weeks experiment all fish were counted and individually weighted to determine the survival, weight and length.

The following parameters were used to evaluate the growth*:*

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 initial weight) /duration] × 100

**Figure 3.7: Length Measurement of Tilapia (left); Weight Measurement of Tilapia Fry (right)**

### 3.8 Economic Analysis

An economic analysis was performed to estimate the net profit from different treatments. The cost of feed and tilapia fry are shown in Table 4.5 & 4.6. The net return/profit was measured by deducting the gross income from the gross cost/treatment. The benefit cost ratio (BCR) was also measured as a ratio of gross income to gross cost.

A simple economic analysis was performed to estimate the net profit. The approximate cost of each ingredient was calculated on the basis of Sylhet local market price (2013). The cost of rice bran was TK. 16/kg, wheat bran was TK. 32/kg, wheat flour was TK. 30/kg, fish meal was TK. 60/kg, maize meal was TK. 27/kg, vit-B complex was TK. 250/kg, vit-E was TK. 20,000/kg and L-arginine was TK. $4\times10^6$/kg. The selling price for tilapia was estimated as TK. 3.50, 3.00, 2.75 and 2.25 as the length and weight were 4.26 cm and 1.187gm, 4.187cm and 1.10gm, 4.1cm and 1.040gm, and 4.00cm and 0.973gm, respectively.

### 3.9 Statistical Analysis

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

# CHAPTER FOUR

# RESULTS AND DISCUSSION

The results obtained from the present investigation are presented in Tables and Figures and discussed character-wise under separate headings in this chapter:

## 4.1 Studies on Determination of Growth Performance

In the present study, L-arginine incorporated in reasonable amount into the food. In both the cases of treated and controlled fish, the same ingredients other than L-arginine in treated group were fed for improvement of growth performances. After digestion of L-arginine, it is subjected to absorption and transport in the blood, and then subsequent assimilation within tissues themselves through the body including brain (improve brain functioning), retina, heart and also increase the secretion of growth hormone.

In the present study, growth of tilapia under the four treatments were estimated on the basis of average final weight and average final length gained by the tilapia fries at the end of the study period and the results so far obtained have been shown in Figures 4.1 and 4.2. Growth performance of *O. niloticus* in terms of weight gain, length gain, SGR and survival rate (%) during the experimentation is shown in table 4.3 and 4.4.

### 4.1.1 Weight

The mean final weight 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 4.1). 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 increases of weight (gm) of tilapia (*O. niloticus*) in different treatment are shown in figure 4.1. Tilapia (*O. niloticus*) fed the control diet (Diet 4) without L-arginine showed the lowest weight gain (WG), and weight gain resultant from the highest level of L-arginine resulted in significantly higher growth for tilapia. The lowest WG was observed in fish fed with Diet 4, which was significantly lower than WG observed for fish fed Diet 3, 2 and 1.

**Table 4.1: 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 | 0.6467±0.0 | 0.9900±0.0 | 1.063 | 1.187±0.03 |
| $T_4$ | 0.31±0.01 | 0.4667 | 0.7500±0.0 | 0.7800±0.0 | 0.900±0.02 | 0.973±0.01 |
| 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

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 were found in $T_3$ which were significantly higher ($p<0.01$) than those of $T_4$, $T_2$ and $T_1$.

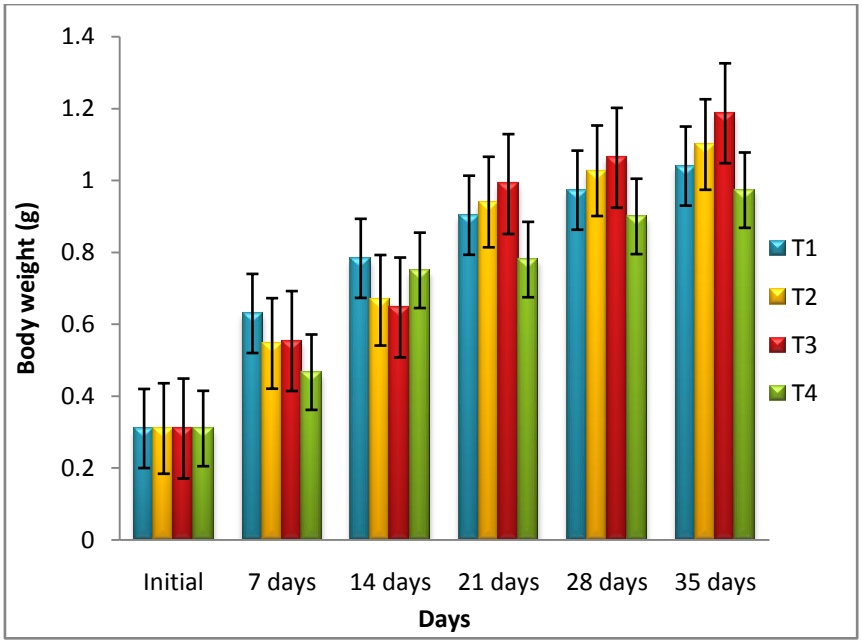


**Figure 4.1: Variation of Body Weight (G) During 35 Days Experiment**

The growth of tilapia significantly varied due to different level of L-arginine. The weight ranged from 0.973 to 1.187 gm. The highest weight (1.187±0.03 gm) recorded was from $T_3$ treatment. The lowest weight (0.973±0.01 gm) recorded was in control treatment ($T_4$). The second highest weight (1.100±0.02 gm) recorded was from $T_2$ treatment. The results revealed that growth increased with increased rate of L-arginine. In present study, tilapia fry showed significant growth improvement when compared to fish fed the control diet ($T_4$). The mean final weight 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.

The highest body weight obtained was 1.187 ± 0.03 gm in treatment $T_3$, which is significantly higher than the other treatment. The body weight obtained in treatment $T_3$, $T_2$ and $T_1$ were significantly higher than $T_4$ which is control. 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. 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). Baños *et al.* (1999) reported the 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. Santiago and Lovell, (1988) studies with Nile tilapia demonstrated that growth depression could occur when fish were fed diets containing an unbalanced dietary arginine levels. In rat, chicken and dogs, decreasing dietary arginine as well as increasing dietary lysine has been reported to depress growth, and this growth depression could be reversed by elevating arginine level in diet (Czarnecki *et al*., 1985; Jones *et al*., 1966, 1967).

### 4.1.2 Length

The mean final length of tilapia (*O. niloticus*) varied 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 4.2). 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 showed 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.

**Table 4.2: 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 | 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

The mean length gain of tilapia (*O. niloticus*) were 1.390 ±0.01 cm, 1.480 ±0.02 cm, 1.553 ±0.04 cm and 1.290 ±0.03 cm in $T_1$, $T_2$, $T_3$ and $T_4$, respectively (Table 4.3). The highest length gain was obtained in $T_3$ (1.553 ±0.04), which was significantly ($p$<0.01) higher than that of $T_2$ (1.480 ±0.02), $T_1$ (1.390 ±0.01) and $T_4$ (1.290 ±0.03). The variation of length and weight of tilapia (O. niloticus) is shown in Appendix 1.

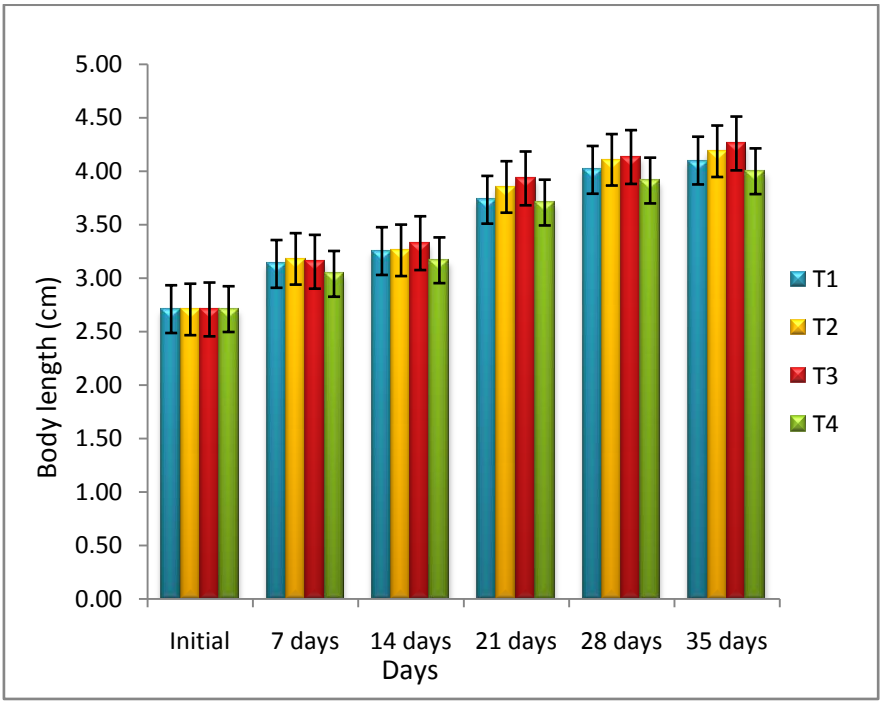


**Figure 4.2: Variation of Body Length (cm) During 35 Days Experiment**

The mean final length of tilapia (*O. niloticus*) varied 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 for 35 days experiment with 0.00143%, 0.0143%, 0.143% and 0.00% L-arginine of supplemental diet, respectively. The highest growth performances in terms of final body weight, length, survival rate and SGR were found in fish fed with supplemental diet with L-arginine of 0.143 g/ 100 g diet ($T_3$).

It is specilated 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.*

2009). Cheng *et al*. (2011) stated that feed efficiency of fish was significantly (Pb0.05) improved by supplementation of glutamine at 2% and the combination of both arginine and glutamine at 1% of diet. In another experiment Kim and Wu. (2004) explained that arginine deficiency represents a major obstacle to maximal growth in piglets. Dietary L-arginine supplementation can augment nitric oxide production and thereby improve vascular health (Preli *et al*. 2002). 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. It is reported that for the production of protein, L-arginine also helps rid the body of ammonia (a waste product) and stimulates the release of insulin (Blum *et al.* 1999). In addition, Chen *et al*. (1999) found that L-arginine stimulates to increase nitric oxide production, which relaxes the blood vessels. Maxwell *et al*. (2002) suggested that L-arginine may benefit certain health conditions. 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 (Han *et al*. 2013). Cheng *et al*. (2011) observed that sufficient weight gain and feed efficiency in red drum fed the diets supplemented with arginine on *Sciaenops ocellatus*. Other studies 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*. 2009), which increase growth hormone responses (Kanaley 2008). All of these results co-relate with the results of present experiment.

**4.2 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 4.3). 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).

**Table 4.3. Growth of 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) |
| $T_1$ | $0.72 \pm 0.02^c$ | $1.390 \pm 0.01^c$ | $231.9 \pm 3.28^c$ | $51.29 \pm 0.18^c$ | $2.07 \pm 0.04^c$ |
| $T_2$ | $0.79 \pm 0.02^b$ | $1.480 \pm 0.02^b$ | $258.5 \pm 3.70^b$ | $54.68 \pm 0.78^b$ | $2.26 \pm 0.04^b$ |
| $T_3$ | $0.88 \pm 0.03^a$ | $1.553 \pm 0.04^a$ | $291.2 \pm 3.91^a$ | $57.38 \pm 1.20^a$ | $2.52 \pm 0.07^a$ |
| $T_4$ | $0.66 \pm 0.02^d$ | $1.290 \pm 0.03^d$ | $217.5 \pm 8.58^d$ | $47.60 \pm 1.12^d$ | $1.90 \pm 0.04^d$ |
| 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= Non significant

The SGR (%/day) as recorded in the present study 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 with 0.00143, 0.0143, 0.143 and 0.00 % L-arginine containing feed. 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.

### 4.3 Survival Rate

The survival rate of tilapia were estimated separately treatment-wise from the sampling data and shown in Table 4.4. Survival rate of fish were high during the feeding trial and significant differences 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 was found in $T_3$ (86.67%) and the lowest in $T_4$ (73.33%). Tilapia fed the control diet ($T_4$) without L-arginine showed the lowest survival rate.

**Table 4.4. Survival Rate (*O. niloticus*) in Aquarium After 35 Days Experimentation**

| Treatment | Survival rate% (M ± SE) |
|---|---|
| $T_1$ | $77.33 \pm 2.31^{bc}$ |
| $T_2$ | $81.33 \pm 2.31^{b}$ |
| $T_3$ | $86.67 \pm 2.31^{a}$ |
| $T_4$ | $73.33 \pm 2.31^{c}$ |
| 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

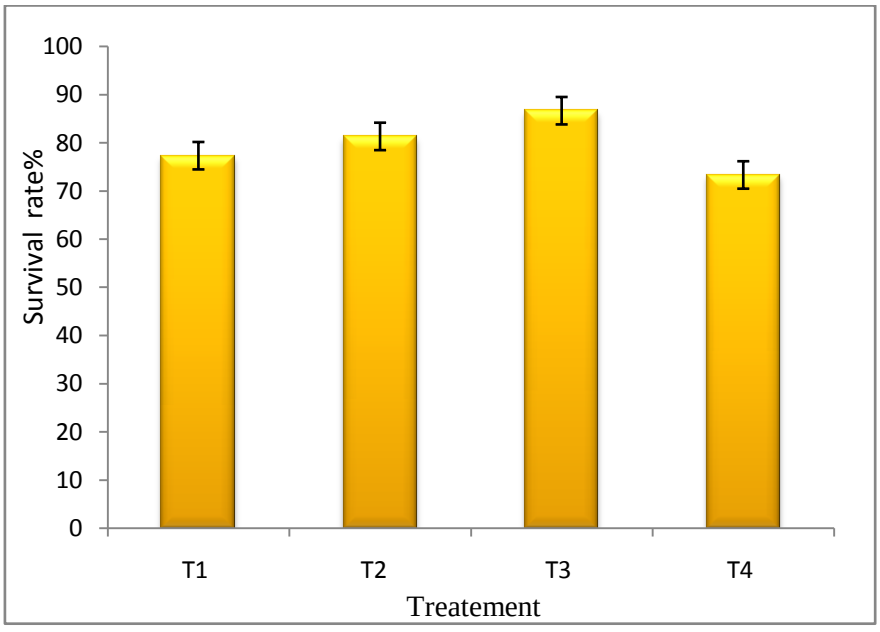


**Figure 4.3: Survival Rate of Tilapia in Four Different Treatments**

The highest survival rate (SR) (86.67%) was observed by the treatement with diet containing 0.143% L-arginine ($T_3$) and were significantly higher than those of treatments $T_1$, $T_2$ and $T_4$. The heighest level of L-arginine showed higher 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 and 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 co-relates with the results of present study.

Dietary supplementation of arginine had beneficial effects on humoral and cell-mediated immune responses of broiler chicks against HPSV (Munir *et al*. 2009). Survival rate during early development can be influenced by development of antigen specific immunity, which was found in early experiment, where arginine supplementation develops antigen specific immunity in mice (Suarez *et al*. 2005) and supplemental arginine (Arg) improved the immunologic response and reduce mortality in rodents with sepsis (Shang *et al*. 2004). Dietary supplementation with 0.8% arginine increased the numbers of white blood cells and granulocytes, and gene expression of interleukin (IL)-8 in spleen (Tan *et al*. 2009). Supplemental arginines (Arg) improved the immunologic response and reduce mortality in rodents with sepsis (Shang *et al*. 2004). Bocchi *et al*. (2000) reported that L-arginine might be used to build the body or immunosystem of the body. This results also support that why the survival rate of tilapia treated with L-arginine was high than that of without L-arginine.

### 4.4 Economic Analysis

The economical analysis of this experiment was done in laboratory condition (in aquarium). There might be some variation if it is done in field condition.

#### 4.4.1 Production of Fish

Total production of tilapia (*O. niloticus*) was (gm/treatment) 18.25 gm, 19.75 gm, 21.93 gm and 16.58 gm in $T_1$, $T_2$, $T_3$ and $T_4$, respectively after 35 days culture period. There were significant variations in production among treatments. The production of $T_3$ was significantly higher than that of $T_1$, $T_2$ and $T_4$.

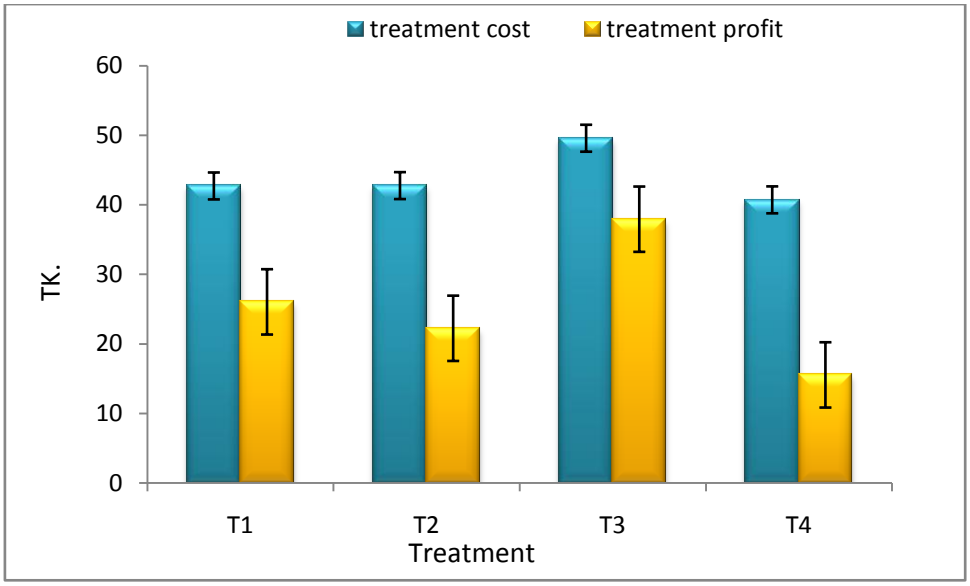


**Figure 4.4: Showing Cost and Profit (TK./Treatment) in Different Treatments**

### 4.4.2 Calculation of Economic Analysis

The main theme of this section is to calculate cost, returns and profitability of fish production as obtained from different treatments. The cost and income calculated for different treatments are presented in Table 4.6.

The cost items e.g. fingerling cost and feed cost and return from the treatments e.g. gross income, net income and benefit-cost ratio (BCR) are discussed below:

**Table 4.5: Feed Cost Calculation**

| **Treatment No. (feed intake in gm)** | | $T_1$ (126) | | $T_2$ (122) | | $T_3$ (124.71) | | $T_4$ (112.23) | |
|---|---|---|---|---|---|---|---|---|---|
| **Ingredients** | **Price Tk/kg** | **%** | **Cost Tk** | **%** | **Cost Tk** | **%** | **Cost Tk** | **%** | **Cost Tk** |
| Rice bran | 16 | 21 | 0.42 | 21 | 0.40 | 21 | 0.43 | 21 | 0.38 |
| Wheat bran | 32 | 15 | 0.60 | 15 | 0.59 | 15 | 0.59 | 15 | 0.54 |
| Wheat flour | 30 | 10 | 0.38 | 10 | 0.37 | 10 | 0.37 | 10 | 0.34 |
| Fish meal | 60 | 40 | 3.02 | 40 | 2.93 | 40 | 2.99 | 40 | 2.69 |
| Maize meal | 27 | 13 | 0.44 | 13 | 0.43 | 13 | 0.44 | 13 | 0.39 |
| Vit-B complex | 250 | 0.50 | 0.16 | 0.50 | 0.15 | 0.50 | 0.16 | 0.50 | 0.14 |
| Vit-E | 20,000 | 0.50 | 12.62 | 0.50 | 12.2 | 0.50 | 12.46 | 0.50 | 11.22 |
| **Total** | | **100** | | **100** | | **100** | | **100** | |
| **Total Cost (Tk)** | | | **17.64** | | **17.07** | | **17.44** | | **15.7** |

**Table 4.6. Economic Analysis of Tilapia (*O. niloticus*) Production**

| Parameters | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|
| Input cost/treatment (in Taka) | | | | |
| Fingerlings cost | 25.00 | 25.00 | 25.00 | 25.00 |
| Feed cost | 4.86 | 4.72 | 4.84 | 4.35 |
| Vit-B | 0.156 | 0.153 | 0.156 | 0.14 |
| Vit-E | 12.62 | 12.2 | 12.46 | 11.22 |
| L-arginine | 0.072 | 0.69 | 7.133 | 0.00 |
| Total cost | 42.71 | 42.76 | 49.57 | 40.71 |
| Return /treatment (in Taka) | | | | |
| Gross income | 68.75 | 75 | 87.5 | 56.25 |
| Net income | 26.04 | 32.24 | 37.93 | 15.54 |
| BCR (Benefit cost ratio) | 1.61 | 1.75 | 1.76 | 1.38 |

### 4.4.3 Gross cost

The total average cost of fingerling was TK. 25/treatment. Here, price of each fingerling was considered TK. 1.00. The price of rice bran was TK. 16/kg, wheat bran was TK. 32/kg, wheat flour was TK. 30/kg, fish meal was TK. 60/kg, maize meal was TK. 27/kg, vit-B complex was TK. 250/kg, vit-E was TK. 20,000/kg and L-arginine was TK. $4\times10^6$/kg. The average total quantity of feed required were 126 gm, 122 gm, 124.71 gm and 112.23 gm in $T_1$, $T_2$, $T_3$ and $T_4$, respectively. The feed costs required in different treatments are shown in Table 4.5. The total costs were TK. 42.71, 42.76, 49.57 and 40.71 for $T_1$, $T_2$, $T_3$ and $T_4$, respectively.

### 4.4.4 Gross income

Gross income was the value of total fish produced. It is calculated by multiplying the total quantity of production by their respective unit quantity market price. The market prices (tk/unit) of the fry were TK. 3.50, 3.00, 2.75 and 2.25 as the length and weight were 4.26 cm and 1.187gm, 4.187cm and 1.10gm, 4.1cm and 1.040gm, and 4.00cm and 0.973gm, respectively. The gross income/treatment was TK. 68.75, 75.00, 87.50 and 56.25 for $T_1$, $T_2$, $T_3$ and $T_4$, respectively.

### 4.4.5 Net income

Net incomes from fish production in different treatments were calculated by deducting gross cost from the gross income. The average total net profit was TK. 26.04, 32.24, 37.93 and 15.54 for $T_1$, $T_2$, $T_3$ and $T_4$, respectively. The net income or profit was significantly higher in $T_2$ than the $T_3$, $T_1$ and $T_4$.

### 4.4.6 Benefit-cost ratio (BCR)

BCR for each treatment was determined as the ratio of gross income to gross cost. The BCR in $T_1$, $T_2$, $T_3$ and $T_4$ were 1.61, 1.75, 1.76 and 1.38, respectively (Table 4.6). The benefit-cost ratio was different in the treatments. The highest BCR was found in $T_3$ (1.76) and the lowest BCR was found in $T_4$ (1.38).

In this experiment among the treatments, $T_3$ showed the highest BCR than those of $T_2$, $T_1$ and T4. It might be due to comparatively better production than the other treatments on the basis of economic analysis.

## 4.5 Water Quality Parameters

Water quality parameters were found to be similar in different treatment. Water temperature ranged from 25 to 29º centigrade, salinity 0.3 to 0.5 ppt, pH7 to7.5, ammonia nitrogen 0.020-0.044 mg/l, dissolved oxygen ≥ 6 mg /litter.

**Table 4.7: Water Parameters in this Experiment**

| Parameter | Range |
|---|---|
| Temperature | 25-29º c |
| Salinity | 0.3-0.5 ppt |
| pH | 7-7.5 |
| Ammonia nitrogen | 0.020-0.044 ppm |
| $DO_2$ | ≥ 6 ppm |

# CHAPTER FIVE

## SUMMARY

Studies on the growth of *Oreochromis niloticus* in term of increase both in length and in weight under four different treatments with different doses of L-arginine as $T_1$ (0.00143%), $T_2$ (0.0143%), $T_3$ (0.143%) and $T_4$ (0.00%) were carried out for a period of 35 days from 23rd July 2013 to 28th August 2013 in twelve aquarium in the laboratory of Fish Biology and Genetics Department, Faculty of Fisheries, Sylhet Agricultural University, Sylhet.

L-arginine has influences on the growth of tilapia and the growth varies with different doses of L-arginine.

The supplemental diet consisting of fish meal 40%, rice bran 21%, wheat bran 15%, maize meal 13%, wheat flour 10%, vit-B 0.5% and vit-E 0.5% was given (with 0.00143%, 0.0143%, 0.143% and 0.00% L-arginine respectively) to all the treatment at a rate of 20% of the total body weight of fries. Weekly sampling was done to adjust feeding rates by measuring the gain in weight of the fries during that period.

The $T_3$ treatment (0.143% L-arg) has the highest survival rate while the treatment $T_4$ (0.00% L-arg) show the lowest survival rate.

The gain in weight, length and SGR exhibited by different treatment were varied significantly. However, the treatment $T_3$ with 0.143% L-arg gave the best growth in term of gain in weight and length and this treatment gave the highest SGR. On the other hand, $T_4$ no L-arg gave the lowest growth in term of gain in weight and length and also the SGR.
The treatment with 0.143% L-arg gave the highest net profit than the other treatments.

# CHAPTER SIX

# CONCLUSION AND RECOMMENDATION

## 6.1 Conclusion

The results from present study confirm the action of arginine in *O. niloticus*. Supplementation with L-arginine also enhanced the feed efficiency of tilapia as well as promoted the development of the intestine. L-arginine elucidate the molecular mechanisms by which arginine regulate growth and other components of the immune system in fish. The present study revealed that L-arginine played a significant role in increasing the growth of tilapia fry. This also helped 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 fry, 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. Incorporation of L-arginine in fish feed improving the fish production, nutritional status and alleviating the poverty of rural people of Bangladesh.

## 6.2 Recommendation

On the basis of information obtained from this study, the following recommendations may be drown:

- The L-arginine treatment of 0.143% of feed may be recommended for successful *O. niloticus* in aquaria culture.
- To improve the production of fish the level of L-arginine should be included.
- Further study should be carried out in different ecological zones to standardize the technology.

## REFERENCES


Alam, M.S., Teshima, S., Koshio, S. and Ishikawa, M. 2002. Arginine requirement of juvenile Japanese flounder Paralichthys olivaceus estimated by growth and biochemical parameters. Aquaculture. 205, 127–140.

Andrew, P.J. and Myer, B. (August 15 1999). "Enzymatic function of nitric oxide synthases". *Cardiovascular Research* 43 (3): 521–531 REVIEW. doi:10.1016/S0008-6363(99)00115-7. PMID 10690324.

Anon. 2009. Arginine hydrochloride injection (marketed as R-Gene 10). FDA Drug Safety Newslette. 2:16-18.

Arana, V., Paz, Y., González, A., Méndez, V. and Méndez, J.D. 2004. Healing of diabetic foot ulcers in L-arginine-treated patients. Biomed Pharmacother. 58:588-97.

Baños, N., Planas, J., Gutierrez, J. and Navarro, I. 1999. Regulation of plasma insulin-like growth factor-I levels in brown trout (*Salmo trutta*). Comparative Biochemistry and Physiology. Part C, Pharmacology, Toxicology & Endocrinology. 124, 33–40.

BBS, 2011.Statistical year book of Bangladesh. Bangladesh bureau of statistics, Statistical division, Government of People's Republic of Bangladesh, Dhaka, 90-110 pp. Hamilton, F., 1822 - Edinburgh & London: 1-405.

Bednarz, B., Jaxa-Chamiec, T. and Maciejewski, P. 2005. Efficacy and safety of oral l-arginine in acute myocardial infarction. Results of the multicenter, randomized, double-blind, placebo-controlled ARAMI pilot trial. Kardiol Pol. 62:421-7.

Blum, A., Porat, R., Rosenschein, U., Keren, G., Roth, A., Laniado, S. and Miller H. 1999. "Clinical and inflammatory effects of dietary L-arginine in patients with intractable angina pectoris." Am J Cardiol. 15;83(10):1488-90, A8.

Bocchi, E.A., Vilella, de., Moraes, A.V., Esteves-Filho, A., Bacal, F., Auler, J.O., Carmona, M.J., Bellotti, G. and Ramires, A.F. 2000. "L-arginine reduces heart rate and improves hemodynamics in severe congestive heart failure." Clin Cardiol. 23(3):205-10.

Buentello, J. A. and Gatlin, D.M. 2000. The dietary arginine requirement of channel catfish *Ictalurus punctatus* is influenced by endogenous synthesis of arginine from glutamic acid. *Aquaculture 188: 311–321.*

Chauhan, A., More, R.S. and Mullins, P.A. 1996. Aging-associated endothelial dysfunction in humans is reversed by L-arginine. J Am Coll Cardiol. 28:1796-804.

Chen, J., Wollman, Y., Chernichovsky, T., Iaina, A., Sofer, M. and Matzkin, H. 1999. "Effect of oral administration of high-dose nitric oxide donor L-arginine in men with organic erectile dysfunction: results of a double-blind, randomized, placebo-controlled study." BJU Int. 83(3):269-73.

Cheng, Z., Buentello, A. and Gatlin, D.M., 2011. Effects of dietary arginine and glutamine on growth performance, immune responses and intestinal structure of red drum, Sciaenops ocellatus Aquaculture 319: 247–252

Cheng, Z., Buentello, A. and Gatlin III, D.M. 2011. Effects of dietary arginine and glutamine on growth performance, immune responses and intestinal structure of red drum, Sciaenops ocellatus. Aquaculture 319: 247–252.

Czarnecki, G.L., Hirakawa, D.A. and Baker, D.H. 1985. Antagonism of arginine by excess dietary lysine in the growing dog. J. Nutr. 115, 743–752.

DoF (Department of Fisheries). 2012-13. *Matshya Pakkhya Saranika*-2013. Department of fisheries. Ministry of Fisheries and Livestock. Government of the People Republic of Bangladesh, Ramna, Dhaka.

DoF (Department of Fisheries). 2011. *Matshya Pakkhya Saranika*-2011. Department of fisheries. Ministry of Fisheries and Livestock. Government of the People Republic of Bangladesh, Ramna, Dhaka.

Dong, J.Y., Qin, L.Q., Zhang, Z., Zhao, Y., Wang, J., Arigoni, F. and Zhang, W. 2011. Effect of oral L-arginine supplementation on blood pressure: a meta-analysis of randomized, double-blind, placebo-controlled trials. *American Heart Journal* 162 (6): 959–965. doi:10.1016/j.ahj.2011.09.012. PMID 22137067.

Fan, Zhou, Shao, Q.J., Jin-xing Xiao, J.X., Peng, X., Ngandzali, B.O., Sun, Z. and Ng, W.K. 2001. Effects of dietary arginine and lysine levels on growth performance, nutrient utilization and tissue biochemical profile of black sea bream,Acanthopagrus schlegelii, fingerlings. Aquaculture 319: 72–80

FAO (Food and Agricultural Organization). 2010. The State of World Fisheries and Aquaculture. FAO., Rome, Italy.

FAO (Food and Agricultural Organization). 2012. The State of World Fisheries and Aquaculture. FAO., Rome, Italy.

Fossel, E.T. 2004. Improvement of temperature and flow in feet of subjects with diabetes with use of a transdermal preparation of L-arginine: a pilot study. Diabetes Care. 27:284-5.

Furuya, W.M., Graciano, T.S., Vidal, L.V.O., Xavier, T.O., Gongora, L.D., Righetti, J.S. and Furuya, V.R.B. 2012. Digestible lysine requirement of Nile tilapia fingerlings fed arginine-to-lysine-balanced diets. R. Bras. Zootec., v.41, n.3, p.485-490,

Gokce, N. 2004. L-Arginine and hypertension. *Journal of Nutrition* 134 (10 Suppl): 2807S–2811S REVIEW. PMID 15465790.

Han, Y. Koshio, S., Ishikawa, M. and Yokoyama,S. 2013. Interactive effects of dietary arginine and histidine on the performances of Japanese flounder Paralichthys olivaceus juveniles.Aquaculture 414–415: 173–182

He, Q., Kong, X., Wu, G., Ren, P., Tang, H., Hao, F., Huang, R., Li, T., Tan, B., Li, P., Tang, Z., Yin, Y. and Wu, Y. 2009. Metabolomic analysis of the response of growing pigs to dietary L-arginine supplementation. Amino Acids. 37(1):199-208. doi: 10.1007/s00726-008-0192-9. Epub 2008 Nov 7.

IUPAC-IUBMB Joint Commission on Biochemical Nomenclature. "Nomenclature and Symbolism for Amino Acids and Peptides". *Recommendations on Organic & Biochemical Nomenclature, Symbols & Terminology etc*. Archived from the original on 29 May 2007. Retrieved 2007-05-17.

Jones, J.D., Petersburg, S.J. and Burnett, P.C. 1967. The mechanism of the lysine–arginine antagonism in the chick: effect of lysine on digestion, kidney arginase and liver transamidinase. J. Nutr. 93, 103–116.

Jones, J.D., Wolters, R. and Burnett, P.C. 1966. Lysine–arginine–electrolyte relationships in the rat. J. Nutr. 89, 171–188.

Jude, E.B., Boulton, A.J. and Ferguson, M.W. Appleton I. 1999. The role of nitric oxide synthase isoforms and arginase in the pathogenesis of diabetic foot ulcers: possible modulatory effects by transforming growth factor beta 1. Diabetologia. 42:748-57.

Kanaley, J.A. 2008. Growth hormone, arginine and exercise. Curr Opin Clin Nutr Metab Care. 11(1):50-4.

Kim, S.W. and Wu, G. 2004. Dietary arginine supplementation enhances the growth of milk-fed young pigs. J Nutr. 134(3):625-30.

"L-Arginine Supplements Nitric Oxide Scientific Studies Food Sources". Retrieved 2007-02-20.

Li, P., Mai, K., Trushenski, J. and Wu, G. 2009. New developments in fish towards functional and environmentally oriented aquafeeds. Amino Acids. 37, 43–53.

Luo, Z., Liu, Y., Mai, K. and Tian, L. 2004. Advance in researches on arginine requirement for fish. A review. J. Fish China. 28: 450-459.

Maxwell, A.J., Zapien, M.P., Pearce, G.L., MacCallum, G., and Stone, P.H. 2002. "Randomized trial of a medical food for the dietary management of chronic, stable angina." J Am Coll Cardiol. 2;39(1):37-45.

Miguez, I., Marino, G., Rodriguez, B. and Taboada, C. 2004. Effect of dietary L-arginine supplementation on serum lipids and intestinal enzyme activities in diabetic rats. J Physiol Biochem. 60(1):31-7.

Moriguti, J.C, Ferriolli, E., Donadi, E.A. and Marchini, J.S. 2005. Effects of arginine supplementation on the humoral and innate immune response of older people. Eur J Clin Nutr. 59(12):1362-6.

Morris, C.R., Morris, S.M., Jr. and Hagar, W. 2003. Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease. Am J Respir Crit Care Med. 168:63-9.

Morris, Jr. and S.M. 2004. Enzymes of arginine metabolism. *The Journal of nutrition* 134 (10 Suppl): 2743S–2747S. PMID 15465778.

Munir, K., Muneer, M.A., Masaoud, E., Tiwari, A., Mahmud, A., Chaudhry, R.M. and Rashid, A. 2009. Dietary arginine stimulates humoral and cell-mediated immunity in chickens vaccinated and challenged against hydropericardium syndrome virus. Poult Sci. 88(8):1629-38. doi: 10.3382/ps.2009-00152.

National Institutes of Health. "L-arginine: MedlinePlus Supplements". November 2010.

Novaes, M.R., Lima, L.A., Ribeiro, J.E., Magalhaes, A.V., Sousa, M.V. and Morhy, l. 2000. Pharmacological effects of arginine supplementation in rats with Walker 256 solid tumor. Arch Latinoam Nutr. 50(3):230-6.

Preli, R.B., Klein, K.P. and Herrington, D.M. 2002. Vascular effects of dietary L-arginine supplementation**.** Atherosclerosis. 162(1):1-15.

Rajapakse, N.W. 2008. Exogenous L-arginine ameliorates angiotensin II-induced hypertension and renal damage in rats. *Hypertension* 52 (6): 1084–1090. doi:10.1161/Hypertensionaha.108.114298. PMC 2680209. PMID 18981330.

Resnick, D.J., Softness, B. and Murphy, A.R. 2002. Case report of an anaphylactoid reaction to arginine. Ann Allergy Asthma Immunol. 88:67-8.

Rytlewski, K., Olszanecki, R., Korbut, R. and Zdebski, Z. 2005. Effects of prolonged oral supplementation with l-arginine on blood pressure and nitric oxide synthesis in preeclampsia. Eur J Clin Invest. 35(1):32-7.

Santiago, C.B. and Lovell, R.T. 1988. Amino acid requirements for growth of Nile tilapia. J. Nutr. 118, 1540–1546.

Schulman, S.P., Becker, L.C. and Kass, D.A. 2006. L-arginine therapy in acute myocardial infarction. The vascular interaction with age in myocardial infarction (VINTAGE MI) randomized clinical trial. JAMA. 295:58-64.

Shang, H.F., Wang, Y.Y., Lai, Y.N., Chiu, W.C. and Yeh, S.L. 2004. Effects of arginine supplementation on mucosal immunity in rats with septic peritonitis. Clin Nutr. 23(4):561-9.

Staff, A.C., Berge, L. and Haugen, G. 2004. Dietary supplementation with L-arginine or placebo in women with pre-eclampsia. Acta Obstet Gynecol Scand. 83:103-7.

Stechmiller, J.K. 2005. Arginine supplementation and wound healing. *Nutrition in Clinical Practice* 20 (13): 52–61 REVIEW. doi:10.1177/011542650502000152. PMID 16207646.

Suarez Butler, M.F., Langkamp-Henken, B., Herrlinger-Garcia, K.A., Klash, A.E., Szczepanik, M.E., Nieves, C. Jr., Cottey, R.J. and Bende. 2005. Arginine supplementation enhances mitogen-induced splenocyte proliferation but does not affect in vivo indicators of antigen-specific immunity in mice. J Nutr. 135(5):1146-50.

Tan, B., Li, X.G., Kong, X., Huang, R., Ruan, Z., Yao, K., Deng, Z., Xie, M., Shinzato, I., Yin, Y. and Wu, G. 2009. Dietary L-arginine supplementation enhances the immune status in early-weaned piglets. Amino Acids. 37(2):323-31. doi: 10.1007/s00726-008-0155-1. Epub 2008 Aug 19.

Tan, B., Yin, Y., Liu, Z., Tang, W., Xu, H.,, Kong, X., Li, x., Yao, K., Gu, W., Smith, B.S. and Wu G. 2011. Dietary L-arginine supplementation differentially regulates expression of lipid-metabolic genes in porcine adipose tissue and skeletal muscle. Journal of Nutritional Biochemistry 22: 441–445

Tapiero, H. 2002. L-Arginine. *Biomedicine and Pharmacotherapy* 56 (9): 439–445 REVIEW. PMID 12481980.

Wilson, A.M., Harada, R., Nair, N., Balasubramanian, N. and Cooke, J.P. 2007. L-arginine supplementation in peripheral arterial disease: no benefit and possible harm. Circulation.116(2):188-95. Epub 2007 Jun 25.

Witte, M.B. and Barbul, A. 2003. Arginine physiology and its implication for wound healing. *Wound Repair and Regeneration* 11 (6): 419–423 REVIEW. doi:10.1046/j.1524-475X.2003.11605.x. PMID 14617280.

Wu, G. 2004. Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. *Journal of Nutritional Biochemistry* 15 (8): 332–451 REVIEW. doi:10.1016/j.jnutbio.2003.11.010. PMID 15302078.

Wu, G.Y. 2009. Amino acids:metabolism, functions, and nutrition. Amino Acids 37, 1–17.

Zhan, Z., Ou, D., Piao, X., Kim, S.W., Liu, Y. and Wang, J. 2008. Dietary arginine supplementation affects microvascular development in the small intestine of early-weaned pigs. J Nutr. 138(7):1304-9.

Zhao, B., Feng, L., Liu, Y., Kuang, S., Tang, L., Jiang, J., Hu, K., Jiang,W.D., Li, S.H. and Zhou, X.Q. 2012. Effects of dietary histidine levels on growth performance, body composition and intestinal enzymes activities of juvenile Jian carp (Cyprinus carpio var. Jian). Aquaculture Nutrition. 18, 220–232.