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Annals of Agricultural Science, Moshtohor (ASSJM)
https://assjm.journals.ekb.eg/

ISSN:1110-0419

Original Article Vol. 62(1) (2024), 77 – 84 DOI: 10.21608/assjm.2024.283918.1275

Effect of dietary L-arginine on growth performance and physiological responses of Nile tilapia, *Oreochromis niloticus*

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Abstract

A feeding trial was conduct to investigate the effect of dietary supplementation of L-arginine on growth performance, feed utilization, hemato-biochemical parameters and hepatic antioxidant enzymes activity of Nile tilapia, *Oreochromis niloticus* for 70 days. Three isonitrogenous and isocaloric diets (310.9 g kg^{-1} crude protein and 18.93 MJ kg^{-1} gross energy) were formulated. Each diet was supplemented with L-arginine at levels; 0.0 (control), 4.0 and 8.0 g kg^{-1} diet. After 70 days the obtained results were as following, the highest weight gain, specific growth rate, protein efficiency ratio and the best feed conversion ratio were recorded in fish fed 8 g L-arginine kg^{-1} diet. As well as, the addition of L-arginine significantly ($P < 0.05$) improved hemoglobin, hematocrit, red blood cells, white blood cells and total protein values compared with the control diet. On the other hand, the addition of L-arginine significantly ($P < 0.05$) decreased values of alanine aminotransferase and aspartate aminotransferase in fish fed 8 g L-arginine kg^{-1} diet. Also, the addition of L-arginine significantly ($P < 0.05$) enhanced the hepatic superoxide dismutase activity and total antioxidant capacity level while; malondialdehyde concentration was significantly ($P < 0.05$) decreased with addition of L-arginine up to 8.0 g kg^{-1} diet. Based on the obtained findings, it could be conclude that the valuable impacts of addition L-arginine up to 8.0 g kg^{-1} diet in enhancement the growth performance, feed utilization hemato-biochemical parameters and hepatic antioxidant enzymes activity of Nile tilapia *O. niloticus*.

Keywords: Tilapia, Amino acids, L-arginine, physiological responses.

Introduction

There is a growing global demand for fish which calls for boosting aquaculture sector (**FAO, 2020**). Functional feed additives are the one of the methods to improve the aquaculture production under intensification **(Elashry *et al.*, 2024)**. Dietary potential supplements such as amino acids, vitamins and herbal plants have been studied for their vital role in enhancing fish performance, health status and immune response of fish (**Lee *et al.*, 2015; Abdel-Tawwab, 2016**). Amino acids have vital metabolic and structural roles (**NRC 2011**) because they are important for protein synthesis, precursors of enzymes, hormones and antibodies which are necessary to meet physiological and immunological processes (**Wilson, 2003; Li et al., 2007; Tejpal *et al.*, 2009; Wu et al., 2013**). According to **NRC (2011)** fish apparently require the same 10 essential dietary amino acids required by most other animals. Among them, arginine which is one of essential dietary amino acids, where its requirement may vary between 1.0 and 3.1% for different species of fish, while for Nile tilapia the arginine requirement is estimated at 1.2% according to **NRC (2011)**. Arginine is not only being an essential amino acid but also a functional amino acid that has various vital functions including; serving as a precursor for the synthesis of proteins, nitric oxide, urea, polyamines, proline, glutamate, creatine and agmatine (**Wu and Morris 1998**) also, stimulation of hormone secretion such as insulin, growth hormone, glucagon and prolactin (**D'mello, 2003**), modulation of some innate immunity mechanisms (**Cheng *et al.*, 2012; Pohlenz *et al.*, 2012; Chen *et al.*, 2016;Wang *et al.*, 2021**) and reduction of oxidative stress (**Wu *et al.*, 2018**). Therefore, the present study was designed to evaluate the effect of the graded levels 0.0, 0.4 and 0.8 g kg^{-1} diet of L-arginine on growth performance, feed utilization, hemato-biochemical parameters and hepatic antioxidant enzymes activity of Nile tilapia, *Oreochromis niloticus* for 70 days.

Materials and Methods

1. Experimental design

The feeding trial was carried out to examine the performance of mono-sex Nile tilapia, *O. niloticus* fed graded levels of L-arginine.

2. Preparation of experimental diets

A basal diet (310.9 g kg^{-1} CP and 18.93 MJ kg^{-1} GE) was formulated (Table 1). L-arginine (LOBA CHEMIE PVT. LTD. India) with graded levels 0.0 4.0 and 8.0 g kg^{-1} were added to basal diet. Therefore, three experimental diets were formulated. Using a pelleting hand noodle maker, all the components (Table 1) were thoroughly combined with L-arginine before being formed into pellets with a diameter of 2 mm. These pellets were then allowed to dry overnight at room temperature for 24 h and were then kept at 4°C.

Table 1. Formulation and proximate composition of the experimental diet (g kg^{-1} diet, dry matter)

Ingredients	%
Soybean meal 44%	490
Corn gluten 62%	120
Yellow corn 8.5%	230
Wheat bran 14%	80
Fish oil	50
Premix[1]	30
Total	
Chemical composition (g kg^{-1})	
Protein	310.9
Lipid	61.75
Ash	45.32
Fiber	48.82
Nitrogen free extract[2]	53.32
Gross energy[3] (MJ kg^{-1})	18.93

[1]Vitamin and mineral mixture kg^{-1} of a mixture contains 4800 I.U. Vit A, 2400 IU cholecalciferol (Vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B12, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. Folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamine. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulphate ($FeSO_4.7H_2O$, 20% Fe), 65 mg; manganese sulphate ($MnSO_4$, 36% Mn), 89 mg; zinc sulphate ($ZnSO_4.7H_2O$, 40% Zn), 150 mg; copper sulphate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I).

[2]NFE (Nitrogen free extract) = 100 − (crude protein + lipid + ash + fibre content).

[3]Gross energy was calculated using gross calorific values of 23.63, 39.52, and 17.15 kj/g for protein, fat, and carbohydrate, respectively, according to **Brett (1973).**

3. Fish rearing technique

Mono-sex Nile tilapia, *O. niloticus* fries (initial weight of 1.5 ± 0.04 g) were purchased from a private farm (El-Sahaba hatchery, Egypt) and acclimated in 10 m^3 concrete pond (4×2×1.25 m) within a greenhouse for two weeks. Fish were fed a commercial feed purchased from Aller Aqua Company with 30 % crude protein and 6 % lipid at a rate of 3% of the total biomass throughout the acclimation period, supplied at equal portions at 9:00, 11:00, and 3:00 p.m. Following acclimation, healthy fish with an average initial body weight of 1.5 ± 0.04 g were randomly stocked in nine plastic tanks (200 L water volume) in triplicates for 70-day. Each group was fed with diet supplemented with 0.0, 4.0 and 8.0 g L-arginine kg^{-1} diet.

Underground water was supplied to each tank housed within greenhouse. About 30% of water volume in each tank was daily replaced by aerated fresh water after removing the accumulated excreta. The amount of feed was calculated on the basis of 3 % of total biomass and offered for experimental fish three times a day, at 9:00 a.m., 11:00 a.m., and 3:00 p.m. Fish were weighed every 15 days to adjust the amount of feed ration. Each week, water samples were taken from each tank in order to measure various aspects of water quality. Using a portable oxygen metre (Jenway, London, UK), the temperature of the water and the amount of dissolved oxygen were measured at the location. A pH-meter (Digital Mini-pH Metre, model 55, Fisher Scientific, Denver, CO, USA) was used to measure the pH. The unionized ammonia (NH3) was measured according to **Boyd** (**1990**) method. Throughout the experiment, the water quality requirements were acceptable and appropriate for Nile tilapia culture **(Boyd, 1990)**. Water quality parameters were monitored daily during experiment period.

4. Growth and feed efficiency

Before the first and second trial, each treatment was counted and the number of fish was recorded. At the end of experiment, all the formulae employed to determine the growth parameters and feed utilization efficiency are listed in the footnote of Table 2.

5. Hemato-biochemical indices

Three fish were used from each tank for each treatment of the trial to collect blood from the caudal vein and were divided into two portions. The first portion of selected fish was collected utilizing 10% EDTA to the estimate haematological parameters **(Hassaan *et al.*, 2020)**. The **Rosenfeld (1947)** approach was used to calculate the differential counting of white blood cells (WBCs). The second portion of selected fish was taken without the use of an anticoagulant, left to coagulate at 4°C, and then centrifuged at 3000 rpm for 10 minutes to obtain the serum. The serum was collected and stored at −20°C until use for measuring the serum biochemical parameters. The procedure outlined by **Reitman and Frankel (1957)** was used to assess the levels serum enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Total serum protein was determined according to **Henry (1964).**

Table 2. Effect of dietary containing different levels of L-arginine on growth performance and feed utilization of Nile tilapia, *O. niloticus* for 70 days

Items	Experimental Diets			± SE	P value
	Control	4	8		
Initial body weight (g fish^{-1})	1.4	1.5	1.5	0.04	0.4687
Final body weight (g fish^{-1})	10.57^{c}	12.23^{b}	13.70^{a}	0.301	0.0039
Weight gain (g fish^{-1})	9.17^{c}	10.73^{b}	12.20^{a}	0.297	0.0047
Specific growth rate (% day^{-1})	2.69^{c}	2.79^{b}	2.95^{a}	0.043	0.0085
Feed intake (g fish^{-1})	15.93^{c}	16.60^{b}	17.40^{a}	0.622	0.0019
Feed conversion ratio	1.73^{a}	1.54^{b}	1.42^{c}	0.027	0.0045
Protein efficiency ratio	1.80^{c}	2.03^{b}	2.19^{a}	0.034	0.0057

Values (± SEM, n = 3). Means in the same row sharing the different superscript are significantly different (P < 0.05). Weight gain (WG) = final weight (g) – initial weight (g); Specific growth rate (SGR) = $LnW_2 - LnW_1/t$ (days), Where, Ln=the natural log; W_1= initial fish weight, W_2 = the final fish weight in grams and t =Period in days; Feed conversion ratio (FCR) was calculated according to by the equation: FCR = Feed intake (g)/weight gain (g); Protein efficiency ratio (PER) = Weight gain (g)/protein ingested (g).

6. Measurements of hepatic antioxidant enzymes activity

Hepatic and gills samples (livers and gills of three fish per replicate) were weighed and homogenized rinsed with ice-cold phosphate buffer (1:10; phosphate buffer: pH 7.4, 0.064 M). Based on the **Peskin and Winterbourn (2000)** method, the homogenate was centrifuged for 10 min at 4°C and 4000 g, and the supernatant was used to assay the activity of superoxide dismutase (SOD). According to **Dogru *et al.* (2008)** the concentration of melanodialdehyde (MDA) was assessed.

2.7. Data statistical analysis

All the obtained data were statistically analyzed by using SAS software (version 9.1) (SAS, 2004). All data submitted to a one-way analysis of variance (One-way ANOVA). Duncan's multiple range test was used to compare differences between treatment means when significant values were observed (**Duncan, 1955**), at (P < 0.05) level.

Results

1. Growth and feed efficiency

Data of Table 2 showed the growth performance and feed utilization of fish fed diet supplemented with L-arginine. Dietary L-arginine with graded levels significantly (P < 0.05) increased the FBW, WG, SGR and PER and the highest value were recorded in fish fed 8 g kg^{-1} diet.

2. Hemato-biochemical indices

Results of the effect of dietary supplementation of L-arginine on hematological parameters of Nile tilapia, *O. niloticus* fed L-arginine were showed in Table 3. the highest levels of hematological parameters; hemoglobin, hematocrit, red blood cells and white blood cells were found in fish-fed diets supplemented with 8 g L-arginine kg^{-1} diet. Results of the effect of dietary supplementation of L-arginine on alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein of Nile tilapia, *O. niloticus* were showed in Table 4. Addition of L-arginine significantly (P < 0.05) decreased the values of ALT, AST where the lowest values of ALT and AST were recorded in fish fed 8 g L-arginine kg^{-1} diet. While, addition of L-arginine significantly (P < 0.05) increased serum total protein whereas, the best value were recorded in fish fed 8 g kg^{-1} L-arginine.

Table 3. Effect of dietary containing different levels of L-arginine on hematology of Nile tilapia, *O. niloticus* fingerlings for 70 days

	Experimental Diets			± SE	P value
Items	Control	4	8		
Hemoglobin (g dL^{-1})	8.67^{c}	10.00^{b}	12.00^{a}	0.351	0.002
Hematocrit (%)	15.00^{c}	19.00^{b}	21.00^{a}	0.582	0.0003
RBC's† (×10^6 µl)	1.56^{c}	1.70^{b}	2.00^{a}	0.0269	0.0004
WBC's‡ (×10^3 mm^{-3})	34.27^{c}	41.67^{b}	43.45^{a}	0.577	0.0001

Values (± SEM, n = 5). Means in the same row sharing the different superscript are significantly different (P < 0.05). †RBCs: Red blood cell counts; ‡WBCs: White blood cell.

Table 4. Effect of dietary containing different levels of L-arginine on biochemical indices of Nile tilapia, *O. niloticus* fingerlings for 70 days

	Experimental Diets			± SE	P value
Items	Control	4	8		
ALT* (UL^{-1})	31.67^{a}	28.00^{b}	25.00^{c}	0.761	0.0005
AST (UL^{-1})**	11.00^{a}	10.00^{b}	9.96^{c}	0.423	0.0026
TP* (gdL^{-1})**	2.90^{c}	3.13^{b}	3.38^{a}	0.053	0.0015

Values (± SEM, n = 3). Means in the same row sharing the different superscript are significantly different (P < 0.05). *ALT: Alanine aminotransferase; **AST, Aspartate aminotransferase; ***TP: Total protein

3. Hepatic antioxidant activity

Results of the effect of dietary supplementation of L-arginine on hepatic antioxidant enzymes activities superoxide dismutase (SOD) total antioxidant capacity (TAC) and melanodialdehyde (MDA) concentration of Nile tilapia, *O. niloticus* fingerlings are presented in Table 5. The activities of hepatic superoxide dismutase (SOD) and level of TAC were significantly ($P < 0.05$) elevated in fish fed 8 L-arginine kg^{-1} On the other hand, MDA concentration was reduced with L-arginine supplementation.

Table 5. Effect of dietary containing different levels of L-arginine on Hepatic antioxidant activities and lipid peroxidation (MDA concentration) (Ug^{-1} protein) of Nile tilapia, *O. niloticus* fingerlings for 70 days

	Experimental Diets			± SE	P value
Items	Control	4	8		
SOD*	82.33^{c}	92.67^{b}	105.00^{a}	1.039	0.0001
TAC**	18.10^{c}	24.00^{b}	28.66^{a}	0.562	0.0001
MDA***	52.43^{a}	50.67^{b}	45.00^{c}	0.749	0.0001

Values (± SEM, n = 3). Means in the same row sharing the different superscript are significantly different (P < 0.05). *SOD: Superoxide dismutase; **TAC: Total antioxidant capacity; *** MDA: Malondialdehyde

Discussion

The present results indicated that L-arginine improved the growth performance and feed utilization of Nile tilapia. Arginine activates adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) to help the body save and use the available energy rather than synthesize lipid, and it also activates the target of rapamycin (TOR) signaling pathway to promote protein synthesis and myogenesis (**Alami-Durante *et al.*, 2020**). Furthermore, dietary arginine significantly increases serum insulin and insulin-like growth factor-I levels (**Pohlenz *et al.*, 2013; Han *et al.*, 2018**). In a similarly, supplementing diets with arginine up to 8.1% for other fish species like atlantic salmon, *Salmo salar* (**Berge *et al.*, 1997**), goldfish juvenile yellow grouper (*Epinephelus awoara*) (**Zhou *et al.*, 2012**), channel cat fish, *Ictalurus punctatus* (**Pohlenz *et al.*, 2014**), juvenile cobia (*Rachycentron canadum*) (**Ren *et al.*, 2014**), Nile tilapia (*Oreochromis niloticus*) (**Yue *et al.*, 2015**) and juvenile blunt snout bream, *Megalobrama amblycephala* (**Liang *et al.*, 2016**) significantly boosted body weight and specific growth rates and feed utilization parameters.

Hematological and serum biochemical in the present study improved with dietary L-arginine. As proven by **Buentello *et al.* (2007)** adding arginine in the diet of channel catfish enables positive effects on both hematological and innate immune responses, such as hematocrit, hemoglobin, phagocytosis and circulating erythrocytes. Subsequently, the current data showing that adding arginine into tilapia diets raised the values of Hb, Htc, RBC's and WBC's, which is in agreement with the previous studies on various fish species including Yellow grouper, *Epinephelus awoara* (**Zhou *et al.*, 2012b**), Red sea bream, *Pagrus major* (**Rahimnejad and Lee 2014**), Jian carp, *Cyprinus carpio* (**Chen *et al.*, 2015**), Yellow catfish, *Pelteobagrus fulvidraco* (**Zhou *et al.*, 2015**) and Nile tilapia, *Oreochromis niloticus* (**Pereira *et al.*, 2017; Vianna *et al.*, 2020**). Also, adding L-arginine increases white blood cell count because it is a precursor of polyamines that are important for cell proliferation and differentiation

(**Buentello *et al.*, 2007**). These results may be explained by the probable role of arginine in promoting fish health by strengthening the immune system's capacity to combat stress and infection by increasing immunological parameters including WBCs because L-arginine is a precursor of polyamines that are important for cell proliferation and differentiation (**Buentello *et al.*, 2007; Vianna *et al.*, 2020**). Nile tilapia fed a diet contained 4 or 8 g L-arginine kg^{-1} have considerably lower AST and ALT activity ($P > 0.05$) in the current findings, which may be favorable for the fish's nutritional state and overall health because L-arginine is a precursor of polyamines that are important for cell proliferation and differentiation (**Buentello *et al.*, 2007**). Similar results were found for ALT and AST activity in Blunt snout bream given diets with L-arginine supplements (**Zhao *et al.*, 2017**).

The present findings indicated that supplementation of L-arginine in the diets of Nile tilapia resulting in a reduction of MDA concentration, while increasing TAC level and activating liver and antioxidant enzymes SOD more strongly. Previous studies reported that arginine promote fish immune response and increase the activity of serum and liver antioxidant enzymes and decrease concentration of MDA (**Buentello *et al.*, 2007; Rahimnejad and Lee, 2014**). In contrast, other studies noted that dietary arginine level had no significant effect on activity of hepatic superoxide dismutase in golden pompano (**Lin *et al.* 2015**). Also, **Zhou *et al.* (2015)** reported that serum SOD, glutathione peroxidase (GPx) activities and MDA concentration of yellow catfish decreased with increasing dietary arginine levels. As well, dietary arginine had no significant effect on the antioxidant abilities in turbot (**Li *et al.*, 2008**); yellow grouper (**Zhou *et al.*, 2012**) and blunt snout bream (**Ren *et al.*, 2013**).

Conclusions

It could be conclude that, using of L-arginine up to 0.8 g kg^{-1} diet improved the the growth performance, feed utilization hemato-biochemical parameters and hepatic antioxidant enzymes activity of Nile tilapia *O. niloticus*. Yet, further studies are actually required for studying the effect of L-arginine on physiological or immune responses of fish and understanding the mechanisms of these effects are also necessity needed.

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تأثير إضافة الأرجنين على أداء النمو والإستجابات الفسيولوجية في أسماك البلطى النيلى (أوريوكرومس نيلوتيكس)

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أجريت تجربة تغذية لدراسة تأثير إضافة الارجنين على أداء النمو والإستفادة من الغذاء وصفات الدم البيوكيميائية ونشاط إنزيمات الكيد المضادة للأكسدة لأسماك البلطى النيلى لمدة 70 يوم. تم تكوين ثلاث علائق متساوية فى محتواها من البروتين والطاقة (310.9 جرام/ كجم بروتين خام و 18.93 ميجا جول/كجم علف طاقة كلية). وتم إضافة 3 مستويات من الأرجنين وهى صفر (الكنترول) و4 و8 جرام أرجنين/كجم عليقة. كانت النتائج المتحصل عليها بعد 70 يوم كالتالى: سجلت الأسماك المغذاه على 8 جرام أرجنين/كجم عليقة أعلى وزن مكتسب ومعدل نمو نسبي ومعدل كفاءة للبروتين كما أعطت أفضل كفاءة لتحويل الغذاء. كذلك أدت إضافة الارجنين إلى تحسين قيم الهيموجلوبين والهيماتوكريت وكرات الدم الحمراء وخلايا الدم البيضاء والبروتين الكلي معنوياً مقارنة بالمجموعة الكنترول. من ناحية أخرى إنخفضت قيم الالنين أمينوترانسفيراز والاسبرتات أمينوترانسفيراز معنوياً في الأسماك المغذاه على 8 جرام ارجنين/كجم عليقة. وكذلك أدت إضافة الأرجنين الى تحسين نشاط إنزيم السوبر أوكسيد ديزميوتيز ومستوى قدرة مضادات الأكسدة الكلية ، بينما إنخفض تركيز المالون داي الداهيد معنوياً مع إضافة الارجنين حتى مستوى 8 جرام أرجنين/ كجم عليقة. بناءً على النتائج المتحصل عليها ، يمكن بيان التأثيرات القيمة لإضافة الأرجينين حتى 8 جرام/كجم عليقة في تحسين أداء النمو وكفاءة الإستفادة من العلف وصفات الدم البيوكيميائية ونشاط إنزيمات الكيد المضادة للأكسدة في أسماك البلطى النيلى.