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Effect of The Amino Acid Taurine on Some Growth Parameters of Grass Carp *Ctenopharyngodon idella* Fingerlings

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Abstract: The objective of this study was to evaluate the effects of taurine, an amino acid additive, on grass carp *Ctenopharyngodon idella* fingerlings (3.16 ± 0.14 gm) growth and feed utilization in glass aquaria for 70 days. Before beginning the feeding experiment, 120 fish were acclimatized for 10days in the laboratories of fisheries department and marine resources, Agriculture College, University of Basrah. Fish fed dietary taurine at levels of 1% (T2), 2% (T3), and 3% (T4) were compared to fish fed a control diet with no taurine (0%, T1). The weight gain (WG) in the T1 was 16.95 g, which was significantly (P≤0.05) higher than the other treatments. T1 had a higher (P≤0.05) mean relative growth rate (RGR) (54.89± 5.66 %) than the other treatments. The specific growth rate (SGR) and daily growth rate (DGR) of T1 were 0.72 ± 0.06 % day $^{-1}$, and 0.28 ±0.01 g, which was also higher (P≤0.05) than other treatments supported with different levels of taurine. Significant differences (P≤0.05) were found in the food conversion ratio (FCR) and food conversion efficiency (FCE) between T1 and the other treatments (T2, T3, and T4). It is clear from the current study that the T1 (0% taurine) better than the other treatments in growth and food utilization indicators. It is concluded from the current study that the addition of taurine (1, 2 and 3%) had inhibited the growth in grass carp fingerlings.

Keywords: Grass carp, Growth, Taurine acid.

Introduction

Amino acids are recognized as important biomolecules, act as raw materials for building proteins, intermediates in various metabolic pathways (Wu, 2010). The proper amino acid balance in fish diets can aid in the development of fish nutrition, which improves the growth and economic returns of fish farms (Li *et al*., 2008). Taurine (2-Aminoethane sulfonic acid) is different from amino acids, does not enter into the proteins building (Ripps & Shen, 2012). Taurine (Tau) may be a conditional amino acid in freshwater fish, during early life stages, such as the larval stage (Zhang *et al*., 2006). It was first described in ox bile (Tiedemann & Gmelin, 1827; Sampath *et al.*, 2020). Taurine is one of the most abundant amino acids in animal tissues, and found in high levels in seafood and meat (Brosnan & Brosnan, 2006). Many vertebrates have the ability to synthesize taurine, Although fish can synthesize taurine in the liver from methionine and cysteine, in some situations, fish are unable to meet the body's taurine requirements and needs a supplementary taurine (Shen *et al*., 2018).

Taurine ($C_2H_7NO_3S$) is an organic osmolyte included in regulation of cell volume, and provides a substrate for the creation of bile salts (Ripps & Shen, 2012). Several studies have found that taurine supplements have numerous benefits for fish, including increased survival, weight gain, protein retention, energy, antioxidants, stress reduction, and disease resistance. Furthermore, plant proteins are taurine deficient, so a plant-based protein food, fish feeding exogenous taurine may be required to maintain physiological functions (El-Sayed, 2013; Sundararajan *et al*., 2014; Sampath *et al*., 2020).

Dietary taurine enhances growth and food efficiency in *Oreochromis niloticus* larvae, that feed on soybean meals, growth rates and food consumption have been significantly improved, with an increase in taurine supplement to 10 g.kg^{-1}, while the growth rate decreases if the percentage of taurine increases more than that, approximately 9.7 g.kg^{-1} of dietary taurine is required, to achieve optimal performance (Al-Feky *et al*., 2015).

Several studies have examined the effect of taurine on the growth of different species of fish, where has the efficacy of taurine supplementation been reported, to improve the growth performance of Japanese flounder *Paralichthys olivaceus*, yellowtail *Seriola quinqueradiata*, sea bass *Dicentrarchus labrax*, cobia *Rachycentron canadum*, European eel *Anguilla anguilla* and guppy *Poecilia reticulata* (Sakaguchi *et al*., 1988; Park *et al*. 2001, 2002; Kim *et al.* 2003; Martinez *et al*. 2004; Kim *et al.* 2005; Matsunari *et al.* 2005; Takagi *et al.* 2005, 2006; Lunger *et al*., 2007; Kim *et al.*, 2008; Takagi *et al*., 2008).

The grass carp *Ctenopharyngodon idella*, belongs to the family Xenocyprididae (Tan & Arbrumster, 2018), this species of fish is considered the first cultured fish in the world, due to its ability to withstand different environmental conditions (Shireman & Smith, 1983). In addition, it is easy to cultivate, provides nutritional requirements and is palatable by the consumer, which lead to the success of its cultivation on a large scale (Pípalová, 2006; Fuller & Benson, 2015). Laboratory experiments were carried out for the cultivation of grass carp fish, the best protein level in processed diets for this fish was 25% (Taher, 2017). Taher *et al.* (2022) studied the effect of some food additives (Thepax and vitamin C) on the growth parameters of grass carp fingerlings.

The study aims to show the effect different levels of taurine on some growth parameters of grass carp fingerlings.

Materials & Methods

Composition of diets

Table (1) shows the feed ingredients used in the manufacture of the feed according to Lovell (1989), the ingredients were grind and passed through a sieve and mixed together, the dietary ingredients were mixed in a feed mixer and moistened with the addition of 50 % (w/v) water (75ºC), then, a mixture of vitamins and taurine was added, it was added at a rate of 0, 10, 20, 30 g.kg^{-1} diet, according to the coefficients T4, T3, T2, T1 respectively. Then the diet was formed using a 50 ml plastic syringe, it was left to dry in the laboratory for two days, placed in plastic bags, and stored in a freezer at 5ºC until used.

Then the chemical analysis of the ration was carried out by Rapid Content Analyzer .The proximate chemical composition of the experiment diets includes 25.35% protein, 3.54% lipids, 11.63% ash, 2.96% fibre, 45.26% nitrogen free extract (NFE) and 11.26% moisture.

Experiment fish

Grass carp fingerlings were used in the experiment, the fish were brought from the Amarah hatchery located in Al-Musharrah District, Maysan Governorate, the experiment was conducted at laboratories of Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah. The grass carp fingerlings were sterilized by saline solution (NaCl) (3%) for three minutes to get rid of parasite and bacterial infection (Herwig, 1979).

Table (1): The components of the diets and the proportions of each component.

Feed Ingredients	Percentages %			
	T1	T2	T3	T4
Fishmeal	25	25	25	25
Soybean meal	22	22	22	22
Wheat meal	29	28	27	26
Wheat bran	20	20	20	20
Starch	2	2	2	2
Vitamins & minerals premix*	2	2	2	2
Taurine	0	1	2	3
Total	100	100	100	100

*Vapcomix (Jordan)

Experiment design

The laboratory experiment was carried out for 70 days between 17 March to 26 May 2021, a total of 120 fish were brought, transferred to the laboratory, acclimatization of fish before starting the experiment for 10 days (including breeding system, diets formulate and the time of food intake) in aquarium, and have been prepared where the fish starved for two days, then they were fed on a laboratory-made control diet.

The initial weight of the fish measured by using a digital scale MH-694 of Chinese origin, for the purpose of starting the experiment. A total of 120 grass carp fish were distributed, 10 fish per tank (three replicates per treatment), on pre-prepared in 12 aquarium (30 × 40 × 60 cm). fishes were fed supplementary diets with different levels of taurine (0, 1, 2, and 3) % for 10 weeks, according to T1, T2, T3 and T4 treatments respectively. Feed was provided at 3% of body weight during the experiment period, the amount of food was given for two meals, the first at half past eight in the morning, while the second was at one o'clock in the afternoon, and six days a week. The feeding was adjusted after weighing the fish every two weeks, for the purpose of follow-up growth, during this period, the water was aerated using air pumps, 20-25% of the water was daily exchanged wire clips to cover the to keep the fish from jumping outside.

Water test

Environmental factors, including water temperature, were measured, by means of a Chinese-origin mercury thermometer, graded from 0-50°C. The dissolved oxygen concentration (mg. l^{-1}), pH values and salinity (PSU) were measured by using a Chinese-origin Water quality meter, model AZ86031.

The growth parameters

The following criteria were calculated according to Macleod (1977), Hepher, (1988) and Jobling (1993):

Weight Gain: WG (g) $= W2 - W1$

Daily Growth Rate (g. day^{-1}):

$$D.G.R = \frac{W2-W1}{T}$$

Specific Growth Rate (%.day^{-1}):

$$S.G.R = \frac{\text{Ln W2} - \text{Ln W1}}{T} \times 100$$

Relative Growth Rate (%):

$$RGR(\%) = \frac{\text{weight gain (g)}}{\text{initial weight (g)}} \times 100$$

Feed Conversion Factor: $F.C.R = \frac{R}{G}$

Feed Conversion Efficiency (%) :

$$FCE = \frac{G}{R} \times 100$$

Satiation Level:

$$\text{Satiation level } \% = \frac{\text{feed intake}}{\text{body weight}} \times 100$$

Where:

W1: Initial weight.

W2: Final weight.

T: The duration of the experiment.

Ln W2: Natural logarithm of final weight w2.

Ln W1: Natural logarithm of the initial weight w1.

R: Dry weight of the feed consumed

G: Weight gain of fish.

Statistical Analysis

Complete Random Design (CRD) was used to study the effect of different treatments on the studied parametres by analysis of variance (ANOVA). The significant differences between the means were compared with LSD under the significance level of 0.05. The program SPSS (Ver. 26) was used in the statistical analysis.

Results

Environmental Factors

Table (2) shows some environmental characteristics of the water used in the growth experiment, where the values indicate the relative stability of these factors throughout the experiment period.

Growth parameters

Table (3) includes some nutritional parameters studied in the growth experiment, it shows the initial and final biomass and the rate of total weight gain of grass carp fingerlings during a 70 day experiment. Significant differences ($P \leq 0.05$) were recorded in the total weight among all the treatments (T1, T2, T3 and T4). Significant increase ($P \leq 0.05$) was observed in the rate of weight gain, relative and specific growth rate as well as, the growth rate in the control treatment (T1) compared to the different treatments (T2, T3 and T4). The statistical analysis of feed conversion rate and feed conversion efficiency in fingerlings grass carp, it showed that there are significant differences ($P \leq 0.05$) between T1 compare with other treatments, while there were no significant differences among T2, T3 and T4, shows the level of satiation in the different treatments, as there are no significant differences between the treatments, the highest level of saturation was in T1 (Table 4).

Table (2): Environmental factors (Means ± Standard Deviation).

Treatment	Temperature (C°)	Dissolved Oxygen ($mg.l^{-1}$)	pH	Salinity (PSU)
T1	28.1 ± 2.01	7.6 ± 0.76	7.5 ± 0.46	2.7 ±0.36
T2	27.9 ± 2.33	7.6 ± 0.74	7.5 ±0.45	2.6 ± 0.21
T3	28.9 ± 2.03	7.6 ± 0.69	7.8 ± 0.38	2.5 ± 0.51
T4	27.7 ± 1.49	7.5 ± 0.65	7.5 ± 0.38	2.5 ± 0.47

Different letters in one column indicate significant differences (P≤0.05).

Table (3): The initial and final biomass, total weight gain (g), relative growth rate (%), specific growth rate ($\%.day^{-1}$) and daily growth rate ($g.day^{-1}$) in fingerlings of grass carp (Means ± Standard Deviation)

Treatment	W1	W2	WG	R.G.R	S.G.R	D.G.R
T1	31 ±1.7	47.95 ± 0.87 a	16.95 ± 0.86 a	54.89 ± 5.66 a	0.72 ± 0.06 a	0.28 ±0.01 a
T2	32 ± 2	44.59 ± 2.46 b	12.59 ± 0.86 bc	39.39 ± 2.25 b	0.55 ± 0.03 b	0.21± 0.01 b
T3	32 ± 1	43.49 ± 1.56 b	11.48 ± 0.57 c	35.87 ± 0.65 b	0.51 ± 0.01 b	0.19 ± 0.01 b
T4	± 0.5 31.5	44.85 ± 1.40 ab	13.35 ± 1.90 b	42.45 ± 6.71 b	0.59 ± 0.08 b	0.22 ±0.03 b

Different letters in one column indicate significant differences (P≤0.05).

Table (4): Feed conversion ratio, feed conversion efficiency and Satiation level in grass carp fingerlings (Means ± Standard Deviation).

Treatment	FCR	FCE (%)	Satiation level %
T1	5.4a ± 0.48	18.46a ± 1.55	3.35a ± 0.53
T2	7.1 b ± 0.37	14.13b ± 0.75	3.11a ± 0.45
T3	7.4 b ± 0.17	13.44b ± 0.32	2.99a ± 0.36
T4	6.8b ± 1.19	15.53b ± 2.10	3.14a ± 0.43

Different letters in one column indicate significant differences (P≤0.05).

Discussion

Environmental Factors

The specifications of the water used in fish farming, it can affect the optimal growth rates of fish and survival rates (Goran *et al*., 2016). Results of the this study indicate that the levels of environmental factors during growth experiments and the level of satiation, it was within the appropriate limits for the cultivation of grass carp, the water temperature ranged between 27.7-28.8°C, dissolved oxygen were 7.5-7.6 $mg.l^{-1}$, pH 7.5-7.8 and salinity 2.5-2.7 PSU.

There was a discrepancy among researchers in determining the optimum water temperature for the growth of grass carp, Pfeiffer & Lovell (1990) mentioned that the optimum temperatures are between 26-30°C, while Diaz *et al*. (1998) between 25.8-30.2°C, As for Hepher (1988), it was shown that the appropriate temperature for cultivating warm water fish ranges between 25-30°C.

Kilambi & Zadinak (1980) stated that the range required to achieve optimal growth, between 19.6-27.2°C, and salinity less than 10 PSU.

Growth parameters

The results of the current study indicated that T1, in which the fish were fed a diet with no taurine supplements, performed better than the other treatments in terms of growth indices (total weight gain, relative growth rate, specific growth rate, daily growth rate, feed conversion ratio and feed conversion efficiency). This indicates that taurine inhibited the growth of fingerlings of grass carp, which could be attributed to the high animal protein content in the experimental diets. The feed used in the current study contains 25% fishmeal, which has a substantial amount of taurine and might be enough to satisfy the nutritional needs of grass carp fingerlings. As a result, the fish will try to excrete taurine that is more than what the body needs, and because fish have a limited ability to precipitate protein, this will result in a high energy cost and a decline in the growth of taurine-supported treatments (Al-Khashali, Pers. Comm.)

The results were consistent with Yang *et al*. (2013), who explained that grass carp fish fed on a diet without taurine supplements displayed the highest performance in comparison to the taurine treatments. It was demonstrated by Abdel-Tawwab & Monier (2018) that dietary taurine increases the activity of the digestive enzyme and increases the antioxidant capacity of common carp, improving both growth performance and health. According to Gaylord *et al*. (2006), dietary taurine enhances rainbow trout growth only when a complete vegetable protein diet is supplied. According to Liu *et al*. (2018), supplementing with taurine at a rate of 10 $g.kg^{-1}$ causes the highest growth in *Scophthalmus maximus* and increases antioxidant capacity; however, consuming 100 $g.kg^{-1}$ of taurine caused damage to the liver and intestines; as a result, increasing the taurine level may be harmful. Lin & Lu (2020) showed that 1 $g.kg^{-1}$ taurine supplementation in a diet high in soybean improved growth and nutrient digestibility in grouper *Epinephelus lanceolatus*.

Kim *et al*. (2008) also reported that taurine supplementation is not necessary for common carp juveniles since there were no noticeable nutritional changes between treatments, on the contrary, taurine supplementation is necessary for juvenile Japanese flounder *Paralichthys* *olivaceus* and contributes to improving the growth of this species as well as raising the contents of taurine in the body and entire tissues with an increase in the nutritional level of taurine. This was explained by the fact that Japanese flounder fish have a lower capability for the biosynthesis of taurine than common carp.

Ferreira *et al*. (2014) studied whether taurine supplementation affected the growth performance of rock bream f *Oplegnathus fasciatus*, fish-fed diets supplemented with taurine (0.5, 1.0, and 1.5 %) exhibited significantly ($P \leq 0.05$) weight gains, specific growth rate, and protein efficiency ratio than fish fed the control diet and 0.25 %. The nutritional efficiency of fish fed on the 0.5% taurine diet was significantly ($P \leq 0.05$) higher than that of fish fed on control, 0.25 %, and 3.0 %, this is contrary to the results of the present study, and it also demonstrates that the diet containing 0.5% was the optimal level of taurine supplementation to promote growth and decrease the fat content of fish bodies.

The results of the present study disagreed with the explanations given by Huang *et al*. (2021) demonstrated that there were no significant differences in growth performance between rainbow trout groups (*Oncorhynchus mykiss*) feeding diets for six weeks with different levels of dietary taurine, their results indicate that the request for taurine in rainbow trout can be met by 30% as fishmeal (containing 0.18% taurine).

The results of this study could not match those of existing studies since different fish species produce a varied amount of taurine depending on their size, food habits, and the activity of the enzyme cysteine sulphinate decarboxylase (CSD). Marine fish like Japanese flounder and Japanese amberjack *Seriola quinqueradiata* exhibit low levels of taurine synthesis due to CSD activities being absent or reduced during the intermediary metabolism of methionine to cystathionine, (Yokoyama *et al*., 2001).

Therefore, taurine supplementation may be necessary, particularly if the fish are fed a plant-based diet. Freshwater fish can produce taurine through the sulfur conversion pathway by increasing the proportion of methionine and cysteine additions (Cowey *et al*., 1992; Kim *et al*., 2008).

Conclusion

The current study expressions that the addition of taurine (1, 2 and 3%) had inhibited the growth in grass carp fingerlings by taking some of the nutritional standards, which was represented by weight gain, relative growth rate, specific growth rate, feed conversion rate, feed conversion efficiency. However, further studies on the effect of taurine on the growth of grass carp are preferred by using a plant-based diet or with reduced animal protein content.

Contributions of Authors

A.S.S: Sample collection and write the manuscript.
F.A.M.S: Statistical analysis and revised manuscript.

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Ethical approval

All ethical guidelines related to Fish and care issued by national and international organizations were implemented in this report.

Conflicts of interest

The authors declare that they have no conflict of interests.

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تأثير الحامض الاميني التاورين في بعض معايير النمو في إصبعيات اسماك الكارب العشبي *Ctenopharyngodon idella*

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المستخلص: هدفت الدراسة الحالية إلى تقييم تأثير مادة التاورين (حامض أميني مضاف) في نمو اصبعيات الكارب العشبي *Ctenopharyngodon idella* (3.16±0.14) واستهلاك الغذاء، في أحواض زجاجية لمدة 70 يوما. قبل بدء التجربة أقلمت الاسمك (120 سمكة) لمدة عشرة ايام في مختبرات قسم الاسماك والثروة البحرية، كلية الزراعة، جامعة البصرة. تمت مقارنة الاسماك التي تتغذى بمستويات مختلفة من التاورين 1% (المعاملة الثانية)، 2% (المعاملة الثالثة) و 3% (المعاملة الرابعة) مع الأسماك التي غذيت على علائق خالية من التاورين 0% (المعاملة الأولى). بلغت الزيادة الوزنية (WG) في المعاملة الاولى 16.95 غم، وكانت اعلى معنوياً ($P\leq0.05$) من المعاملات الأخرى. معدل النمو النسبي (RGR%) في المعاملة الاولى(54.89±5.66) قد ارتفع معنويا ($P\leq0.05$) عن المعاملات الاخرى. معدل النمو النوعي (SGR) ومعدل النمو اليومي (DGR) في المعاملة الاولى (0.72 ±0.06% يوم $^{-1}$، و 0.28 ± 0.01 غم) قد ارتفعت معنويا ($P\leq0.05$) عن المعاملات الأخرى المدعمة بمستويات مختلفة من التاورين. وجدت فروق معنوية ($P\leq0.05$) في نسبة التحويل الغذائي (FCR) وكفاءة التحويل الغذائي (FCE) بين المعاملة الاولى والمعاملات الأخرى. يتضح من الدراسة الحالية إن المعاملة الاولى (0% تاورين) أفضل من المعاملات الأخرى في مقاييس النمو واستعمال الغذاء. يستنتج من ذلك أن إضافة التاورين (1، 2 و3)% عملت على تثبيط النمو في إصبعيات الكارب العشبي.

الكلمات المفتاحية: الكارب العشبي, النمو, حامض التاورين.