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Effects of Creatine and Guanidinoacetic Acid as Feed Additives on Nile Tilapia (*Oreochromis niloticus*) Growth Performance

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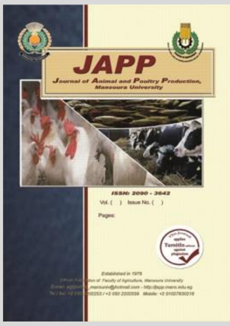

ABSTRACT

This study was designed to investigate the effect of Creatine (Cr) and Guanidinoacetic acid (GAA) as feed additives on growth performance, feed utilization and body composition of sex reversed (male) Nile tilapia (*Oreochromis niloticus*) fingerlings. Creatine and Guanidinoacetic acid play an important role in metabolism and can be applied in aquaculture for improving the growth performance, feed utilization and carcass composition of aquatic animals. Nile tilapia (10.4±0.4 g / fish) were fed a commercial diet (30% crude protein and gross energy 4120.7 Kcal/kg.) supplemented with different additives of Cr and GAA (0.8 and1.2g / kg diet). Fish were randomly distributed (in triplicates) into 5 treatments (control, Cr 0.8, Cr 1.2, GAA 0.8 and GAA 1.2 g/ kg diet). Treatments were performed in 15 concrete ponds (1x4 m each). Fish were fed three times a day for 12 weeks at 4% of their body weight. The optimum growth performance, feed utilization and body composition were obtained at 1.2g GAA/ kg diet. The GAA in Nile tilapia diet was promising for enhancing the growth performance in Nile tilapia which may improve fish production. Using GAA is beneficial in aquaculture, definitely on Nile tilapia farming.

Keywords: Creatine, Guanidinoacetic acid, Nile tilapia, growth performance, feed utilization and body composition.

INTRODUCTION

With growing aquaculture industry and production almost 12-fold over the last three decades (FAO, 2016), the request of fishmeal (FM) increased. Hence, the price of fish meal increased and became unusual and unstable for supply. That situation forced the researchers to discover another substitute for FM for balanced diets formulation, nutritionally and economically at the same time (Hardy, 2010).

At the present, many types of protein sources inclusive plant proteins, single cell proteins and animal by-products have been evaluated as FM alternatives (Zeng et al., 2017), via decreasing the amount of fish meal used for aqua feeds without reducing growth performance or effects on health case of aquaculture organisms which effects on more development and more useful aquatics farming (Tidwell et al., 2005). Soybean (SM) has consider the most promising plant protein source. Also, use of SM in aqua feed faced many problems because of the imbalance in the profile of amino acids (Floreto et al., 2000), worse palatability and attendance of several anti- nutritional factors (Anderson & Wolf 1995). Thus, the lack of some nutrients and bioactive compound in plant protein sources comparing with FM led to deficiency and decreasing performance (Barrows et al., 2010).

Creatine has a great role in the cell as an energy buffer. As the energy system is a basic part of the organism (Borchel *et al.*, 2014), it is a semi-essential nutrient derivative from the amino acids methionine, arginine and glycine (Teixeira *et al.*, 2017). A large amount of animal proteins, like rich sources of creatine, should be involved in the feed formulation to avoid creatine lack (Zeng *et al.*, 2017). Therefore, animal by-products, which are rich in Cr, It accounted for a large portion of the stake, no marks for a Cr shortage could be discovered. With the absence of animal protein in plant protein diets, the hazard for a Cr shortage increased (Ringel *et al.*, 2007).

Guanidinoacetic acid (GAA), A very important mediator in biosynthesis of Cr, is an enzyme-catalyzed step from L-arginine and glycine or get it from foods rich in animal protein (Ostojic *et al.*, 2014). It is assumed that around 2/3 to 3/4 of the daily requirement is synthesized by *de-novo* however the reminder It should be provided by feed (Ringel *et al.*, 2007). At the present time, supplemental GAA is used as a feed additive for pigs and broilers to enhance breast meat yield, growth and the feed conversion ratio. It is an effective alternative to dietary arginine in immature chicks which enhances reproductive indicators and postnatal progeny performance in quails (Ostojic, 2016). However, there is no on hand investigation on surveying the effects of Cr or GAA in plant-based diets for fishes like tilapia (Zeng *et al.*, 2017).

The fast growth of tilapia production all over the world needs more costly input, with a lot of dependence on formulated diets (El-Sayed, 2006). Therefore, finding novel functional feed supplements have been a major dare facing tilapia feed industry (Hanan *et al.*, 2017).

The aim of this study was to investigate the effect of Cr or GAA supplementation in all-plant protein diets on growth performance, feed utilization and carcass composition of juvenile Nile tilapia (Oreochromis niloticus).

MATERIALS AND METHODS

Experimental Design

This experiment was carried out at the Fish Experimental Unit of the Department of Animal Production, Faculty of Agriculture, Al-Azhar University,

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Cairo, Egypt. Tilapia fish (*O. niloticus*) were randomly distributed into 15 concrete ponds (1x1x4m) diameters (80 fish/ pond – 20 fish /m^3).

All experimental ponds were supplied with dechlorinated tap water through a water pipeline system and were supplied with air through air pipeline using air blower 5 HP. The water was renewed at a rate of 30% every 24 hours. Fish feces and feed wastes were removed everyday by siphoning. The study lasted for 12 weeks after two weeks` adaptation.

Experimental Fish

Apparently healthy sex-reversed (all male) Nile tilapia (*O. niloticus*), fingerlings were purchased from Abbasa Research Station, Abo Hammad, Sharkia Governorate, Egypt. The fingerlings were transported at the morning using a special fish transport car with aeration tools. Fingerlings were adapted to the experimental condition for 15 days before starting the experiment.

At the end of the acclimation time, a random sample of fish was netted from each pond. The average initial weights were recorded. Different parameters of water quality as water temperature, ammonia (NH_4–N), pH and nitrites (NO_2–N) were measured every 2 weeks by Hanna Instrument, Inc., Jud-Cluj Romania. The average values of these parameters throughout the study were; T = 27.5 ±1°C, DO = 6.7 ±1.2 mg/l, NH_4–N = 1.14 mg/l, NO_2–N = 1.14 ± 0.14 mg/l and pH = 7.75 ± 0.20. They were in the acceptable ranges for Nile tilapia (*Oreochromis niloticus*) culture in all treatments.

Experimental Diet

Nile tilapia (10.4 ± 0.4 g / fish) were fed a commercial diet (30% crude protein and gross energy 4120.7 Kcal/kg.) enhanced with different additives of Cr and GAA (0.8, 1.2g / kg diet). Fish were randomly distributed (in triplicates/treatment) into 5 treatments (control, Cr 0.8, Cr 1.2, GAA 0.8 and GAA 1.2 g/ kg diet). Creatine was bought from local market and GAA was Cre-Amino® from Evonik Company).

Fish of all treatments were fed a commercial diet be formed of local ingredients, mainly fishmeal 17.0%, soybean meal 30.0%, yellow corn 15.0%, wheat bran 20.0%, alfalfa hay 12.5%, sunflower oil 3.0%, minerals mixture 0.5%, vitamin mixture 1.0% and carboxymethyl cellulose 1.0%. The chemical composition of the diet was crude protein 30%, ether extract 4.68%, crude fiber 6.42% and gross energy 4120.7 Kcal/kg. Each one kg of vitamin mixture contained: Vitamin A 16060 IU, B1 6 mg, B3 1500 IU, B6 9 mg, B12 6 mg, C 60 mg, E 60 mg, pantothonic acid 60 mg, nicotinic acid 120 mg, folic acid 6 mg, biotin 0.3 mg and choline chlorids 30 mg. Fish were fed at the rate of 4% of wet body weight per day and it offered three times at 8.00, 12.00 and 17.00 hours. The fish in each pond were weighed biweekly and the feed weight was adjusted after each fish weighing (according to the manufacturer the Aller Aqua Egypt Industrial Company located in 6th October City).

Proximate analysis of fish

Fish samples were collected randomly at the start and at the end of the trial for whole body analysis consist of moisture, crude protein, crude fat and total ash, contents were determined according to Association of Official Analysis Chemists (AOAC, 2006) methods. These samples were kept frozen at -4°C till the time of analysis (Eya and Lovell, 1997).

Measurement of Fish Growth and Feed Utilization

All fishes were separately weighed to the nearest 0.1 g at the beginning of the experiment and at once every two weeks intervals throughout the experimental period. The mortality of fish was recorded biweekly at the time of weighing. The growth performance and feed utilization efficiency were calculated as following:

Body weight gain (BWG) = final weight (g/fish) – initial weight (g)

Daily weight gain (DWG) = body weight gain (BWG) / period.

Survival rate (SR) % = (No. of fish survived at the end of the experiment / whole number of fish at the beginning) ×100.

Feed conversion ratio (FCR) = feed intake (g) / body weight gain (g)

Feed efficiency ratio (FER) = body weight gain (g) / feed intake (g)

Protein efficiency ratio (PER) = body weight gain (g) / protein intake (g)

Statistical analyses:

The data were subjected to one-way analysis of variance (ANOVA) at a 95% confidence limit, using Statistical Analysis System (SAS) program (SAS, 2002). Differences among means were tested using Duncan's Multiple Range (Duncan, 1955).

RESULTS AND DISCUSSION

RESULTS

Average values of initial weight, final body weight, body weight gain and daily weight gain of Nile tilapia fingerlings fed different levels of Cr or GAA are shown in Table 1. The initial weight was similar in all treatment groups with no significant differences (P > 0.05). In the present study, means of body weight at the start of the experimental period indicate that the distribution of individual fish between the experimental treatment was completely random. Final body weight, body weight gain and daily weight gain were increased significantly (P<0.01) by 1.2g / kg diet GAA treatment (115.66, 105.33 and 1.26g), respectively; followed by 1.2 g / kg diet Cr treatment (100.33, 90.02 and 1.07g), respectively, then 0.8 g /kg diet GAA treatment (91.33, 81.02 and 0.96g), respectively. While there were no significant differences (P>0.05) between control (83.33, 73.02 and 0.87 g), respectively and 0.8 g / kg diet Cr treatment (83.67, 73.34 and 0.87g), respectively.

Table 1. The Effect of different additives of Creatine and Guanidinoacetic acid (GAA) on growth performance of Nile tilapia fingerlings reared in concrete ponds.

Treatment	IW	FBW	BWG	DWG
Control	10.31±0.38	83.33±1.86^d	73.02±1.8^d	0.87±0.22^d
Cr 0.8 g / kg diet	10.33±0.45	83.67±1.85^d	73.34±1.8^d	0.87±0.22^d
Cr 1.2 g / kg diet	10.31±0.43	100.33±4,4^b	90.02±2.4^b	1.07±0.29^b
GAA 0.8 g / kg diet	10.67±0.44	91.33±2,3^c	81.02±2.3^c	0.96±0.29^c
GAA 1.2 g / kg diet	10.34±0.44	115.66±2.3^a	105.33±2.4^a	1.26±0.29^a

Values in the same column with different superscripts are significantly different at p<0.01. IW= initial weight, FBW=final body weight, BWG= body weight gain and DWG= daily weight gain.

Feed intake (FI), protein intake (PI), feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) are given in Table 2. There was a significant difference (P<0.01) among different supplementation treatments in FI and PI. The highest FI and PI were noticed in 1.2 g GAA / Kg diet treatment (161.7 and 48.5 g), respectively. While there were no significant differences (P>0.05) between 1.2 g /kg diet Cr treatment (155 and 46.5 g), respectively and 0.8 g / kg diet AAG treatment (157.3 and 47.3g), respectively. The least FI and PI values were recorded in control and 0.8 g / kg diet Cr treatment (157 and 47.1 g) and (155 and 46.5 g), respectively.

Feed conversion ratio FCR recorded the lowest value 1.53 in 1.2 g / kg diet AAG treatment followed by1.72 in 1.2 g / kg diet Cr treatment, then 1.94 in 0.8 g / kg diet AAG treatment. On the other hand, FCR recorded the highest value in control and 0.8 g / kg diet Cr treatment (2.15 and 2.13), respectively with non-significant differences (P>0.05). FER and PER recorded the highest significant (P<0.01) values (0.65 and 2.17), respectively in 1.2 g / kg diet AAG treatment followed by (0.58 and 1.94), respectively in 1.2 g / kg diet Cr treatment, then (0.52 and 1.72), respectively in 0.8 g / kg diet AAG treatment. On the other hand, FER and PER recorded the lowest values in control (46 and 1.55) and 0.8 g / kg diet Cr treatment (47and 1.58), respectively with non-significant differences (P>0.05).

The previous results clearly proved that the dietary supplementation of the basal diet fed to *O. niloticus* with the feed additive GAA significantly enhanced the growth performance and feed utilization for Nile tilapia at 1.2g /kg diet AAG treatment compared to the other treatment.

Table 2. The Effect of different additives of Creatine and Guanidinoacetic acid (GAA) on feed utilization of Nile tilapia fingerlings reared in concrete ponds.

Treatment	FI g / fish	PI g / fish	FCR	FER	PER
Control	157.0±1.53[b]	47.1±0.46[b]	2.15±0.32[a]	0.46±0.01[d]	1.55±0.26[d]
Cr 0.8 g / kg diet	155.0±1.53[b]	46.5±0.46[b]	2.13±0.55[a]	0.47±0.12[d]	1.58±0.40[d]
Cr 1.2 g / kg diet	155.0±1.53[ab]	46.5±0.46[ab]	1.72±0.61[c]	0.58±0.20[b]	1.94±0.67[b]
GAA 0.8 g / kg diet	157.3±2.0[ab]	47.2±0.61[ab]	1.94±0.34[b]	0.52±0.01[c]	1.72±0.28[c]
GAA 1.2 g / kg diet	161.7±1.3[a]	48.5±0.40[a]	1.53±0.33[d]	0.65±0.15[a]	2.17±0.47[a]

Values in the same column with different superscripts are significantly different at p<0.01. FI= feed intake PI protein intake FCR= feed conversion ratio FER= feed efficiency ratio PER= protein efficiency ratio

Average values of dray mater (DM), crude protein (CP) ether extract (EE) and Ash of Nile tilapia fingerlings fed different levels of Cr or GAA are shown in Table 3. The DM was similar in all treatment groups with no significant differences (P>0.05). CP was increased significantly (P<0.01) by 1.2g / kg diet GAA treatment (68.37%) followed by 1.2 g / kg Cr treatment (66.53%) then 0.8 g /kg diet GAA and 0.8 g / kg diet Cr treatment. (64.53 and 63.83%), respectively. CP recorded the lowest value in the control (61.2%).

Ether extract (EE) indicated the lowest values (P<0.01) 17.3% in 1.2g / kg diet GAA treatment. While EE recorded the highest significant (P<0.01) values 19.37 in control, followed by (18.40, 18.37 and 18.03%) in 0.8 g / kg diet Cr, 0.8 g / kg diet GAA and 1.2 g / kg diet GAA treatments, respectively then (0.52 and 1.72%), respectively in 0.8 g / kg diet AAG treatment respectively. As for Ash recorded the highest significant (P<0.01) values 19.43% in control; followed by (17.77and 17.10%) in 0.8 g / kg diet Cr and 0.8 g / kg diet GAA treatments, respectively then (15.43 and 14.60%), respectively in 1.2 g / kg diet Cr and 1.2 g / kg diet AAG treatments, respectively.

Table 3. The Effect of different additives of Creatine and Guanidinoacetic acid (GAA) on chemical composition of Nile tilapia fingerlings reared in concrete ponds.

Treatment	DM	CP %	EE %	Ash %
Control	28.31±0.03	61.20±0.20[d]	19.37±0.09[a]	19.43±0.28[a]
Cr 0.8 g / kg diet	28.45±0.05	63.83±0.19[c]	18.40±0.35[ab]	17.77±0.43[b]
Cr 1.2 g / kg diet	28.25±0.03	66.53±0.69[b]	18.03±0.42[ab]	15.43±0.34[c]
GAA 0.8 g / kg diet	28.23±0.04	64.53±0.59[c]	18.37±0.09[ab]	17.10±0.55[b]
GAA 1.2 g / kg diet	28.21±0.02	68.37±0.48[a]	17.03±0.47[c]	14.60±0.06[c]

Values in the same column with different superscripts are significantly different at p<0.01. DM=dray mater, CP =crude protein and EE= ether extract.

DISCUSSION

The advance of growth performance concomitantly with the supplementation of the commercial GAA comparing with Cr may be attributed to the fact that even though creatine as a feed supplement play an important work for ideal growth and nutrient utilization in human (Semeredi *et al*., 2019) and different species of farm animals like pigs (Michiels *et al*., 2012), chicken (Ringel *et al*., 2007) and poultry meat quality (Stahl *et al*., 2003). It is a fact that around 95% of Cr pool is placed in muscle tissue (Wyss and Kaddurah-Daouk, 2000). Though, synthesis of Cr in the kidney and liver is not enough for ideal supply of the animals and show some drawbacks like instability through storage and at lower pH value due to its chemical properties and high expense, compared with GAA which is more stable and less expensive (Baker, 2009). The oral delivery is potentially a good approach because the compound is rapidly absorbed from the gastrointestinal tract, being transformed into Cr which protects the amino acids involved (arginine & glycine) in its synthesis (Ostojic *et al.*, 2015).

In agreement with the present results, Zeng *et al.* (2017) realized that dietary supplementation of 0.4g GAA kg^{-1}diet to the soymeal diet significantly improved growth performance of bullfrog Rana (*Lithobates*) these results may be attributed to enhancing in creatine synthesis which subsequently leads to additional energy for cellular bioenergetics (Ostojic, 2016). In the same way, it has been showed that creatine reasons of cell hydration resulting in increased total body water and cell volume and, lastly increased volume of muscle (Haussinger, 1996). Bekara *et al.* (2007) found that cells hydration facilitates protein synthesis, prevent protein decomposition and improves synthesis of glycogen.

Fu *et al.* (2015) found a decreasing in feed conversion ratio significantly in Jian carp (*Cyprinus carpio*) when supplemented with 0.5g GAA kg^{-1} diet. Wang *et al.* (2012) and Mousavi *et al.* (2013) expected that increasing the amount of guanidine acetic acid in the diet would improve performance in the animals at all periods which contribute to Cr formation and would conserve arginine, which could then be used by the body for other functions such as protein anabolism. Similarly, Dilger *et al.* (2013) concluded that supplemented broiler diets with GAA had a good response.

That may be attributed to the sparing effect of arginine and glycine, thus delivering more glycine and arginine available for body protein or endogenous amino acids synthesis, leads to improved body growth (Zeng *et al*., 2017).

Michiels *et al*. (2012) their data suggesting that supplementing (0.6 and 1.2g kg^{-1}) GAA in whole vegetable diets develops performance and carcass properties in terms of the gain: feed ratio and the breast meat yield. Moreover, Lemme *et al*. (2007b) found linear increases of muscle creatine levels with increasing dietary GAA. In this respect and agreed with our results on Juvenile *O. niloticus* supplemental GAA can be seen as an efficient creatine source particularly when compared to the positive control and the creatine supplemented treatments.

In contrast with the present results, Teixeira *et al.* (2017) found that the use of GAA did not encourage improvements in performance or blood creatinine level in piglets in nursery phase. Lemme *et al.*(2007a) reported significant improvements in FCR of up to five and seven points, respectively, in 42 days old broilers. In contrast with the present findings, Zeng *et al.*(2017) found no significant differences in whole body composition were detected among dietary treatments (0.2, 0.4, 0.6 and 0.8 GAA g kg^{-1} diet in a soymeal diet. In addition, Fu *et al.* (2015) also fed Jian carp GAA- containing diets. However, in agreement with the present results, Fang *et al.*(2016) described that total replacement of fishmeal by soymeal leads to significant decrease of whole-body protein and increasing of lipid in bullfrog.

The conflicting results could be attributed to the differences in dietary formulation and experimental conditions (Zeng *et al*., 2017). It is worth to note that, arginine in mammals is ordered as a semi-essential or conditionally essential amino acid (Baker, 2009). It is an intermediate of the urea cycle and can be synthesized from citrulline (Wang *et al.*, 2006). However, in fish and shrimp, arginine has been shown to be an essential amino acid due to the very poor activity of the urea cycle (NRC, 2011). So, the effective and harmless amount of GAA as a dietary additive for broilers has been suggested to be lower than 0.6-0.8g kg^{-1} diet by European Food Safety Authority (2009). Similarly, Zeng *et al.* (2017) suggested that dietary GAA dose for bullfrog should be kept less than 0.4 g kg^{-1} as higher GAA levels negatively affected bullfrog performance.

CONCLUSION

The results of our study show that adding 1.2 g/ kg diet of Guanidinoacetic acid (Cre-Amino) in Nile tilapia diets improves growth performance, feed utilization and body composition of Nile tilapia (*Oreochromis niloticus*) and that it is a good alternative for natural creatine.

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أثار الكرياتين وحمض الجوانيدين أسيتيك كإضافات غذائية على أداء نمو البلطي النيلي

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صُممت هذه الدراسة لتوضيح أثار الكرياتين وحمض الجوانيدين أسيتيك كإضافات غذائية في علائق البلطي النيلي على أداء النمو والاستفادة الغذائية وتركيب جسم إصبعيات أسماك البلطي النيلي وحيد الجنس (ذكور). الكرياتين وحمض الجوانيدين أسيتيك يلعبان دورًا مهمًا في عملية الأيض الغذائي، ويمكن تطبيق ذلك في الاستزراع المائي لتحسين أداء النمو والاستفادة من الغذاء وتركيب جسم الحيوانات المائية. تم استخدام أسماك البلطي النيلي (١٠,٤ ± ٠,٤ جم/ سمكة)، فغُذيت على علائق تجارية (٣٠% بروتين خام وطاقة كلية ٤١٢٠,٧ كيلوكالوري/ كجم)، وتمت إضافة الكرياتين وحمض الجوانيدين أسيتيك (ج أ أ) بنسب ٠,٨ و١,٢ جم / كجم عليقة. تم توزيع الأسماك عشوائيًا في خمس معاملات لكل معاملة ثلاث مكررات (كنترول، ٠,٨ كرياتين، ١,٢ كرياتين، ٠,٨ ج أ أ ، ١,٢ ج أ أ جم / كجم عليقة). أجريت هذه المعاملات في ١٥ حوض خرساني أبعاد كل منها ٤x١ م. تم تغذية الأسماك ثلاث مرات يوميًا لمدة ١٢ أسبوعا، بمعدل ٤% من وزن الأسماك يوميا. ومن أهم النتائج المُتحصّل عليها وُجد أن أداء النمو والاستفادة الغذائية وتركيب الجسم الأمثل تم الحصول عليه في المعاملة ١,٢ ج أ أ جم / كجم عليقة. وعليه فإن إضافة حمض الجوانيدين أسيتيك في علائق البلطي النيلي كان له تحسين واعد في معدل نمو أسماك البلطي النيلي والذي قد يزيد من الإنتاج السمكي. وباختصار فإن استخدام حمض الجوانيدين أسيتيك مفيد في الاستزراع المائي وخصوصًا في مزارع البلطي النيلي.