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Influence of Guanidinoacetic Acid Supplementation of Fish Diets with Different Levels of Energy Content on Growth Performance and Serum Metabolites

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Abstract

Objective: This research was performed to explore the impacts of guanidinoacetic acid (GAA) supplementation of fish diets with variable energy contents on the growth performance and blood serum parameters of Nile tilapia fish.

Methodology: Nile tilapia Juveniles with an average weight of 20 g were cultured in glass aquaria and fed four formulated pelleted diets for 60 days. Our experiment was divided into four experimental groups: the first group **(G1)** was fed a basic control diet without any GAA supplementation. The other experimental groups, **G2, G3** and **G4,** were fed diets with different levels of energy (25, 50 and 75 kcal/kg less than in the control, respectively) and supplemented with 0.06% GAA.

Results: The fish in G2 and G3 had significantly higher final body weights (FBWs) and body weight gains (BWGs) than those in the other experimental groups. In addition, the fish in G4 had significantly lower (BWGs) and protein efficiency ratio (PERs) than those in the other experimental groups. The serum levels of glucose and urea in G4 fish were significantly higher than those in fish in the other experimental groups. No significant differences in liver superoxide dismutase (SOD) or nitric oxide (NO) levels were observed among the experimental groups.

Conclusion: A reduction in digestible energy by up to 50 kcal / kg with supplementation of GAA at a level of 0.06% improved the growth performance of Nile tilapia fish.

Keywords: Nile Tilapia, Guanidinoacetic acid, Energy, Performance, Serum metabolites.

List of abbreviations: Arg: Arginine; Cre: Creatine; AGAT: Amidinotransferase; GAMT: Guanidinoacetic Methyl Transferase; PCr: Phosphocreatine; FBW: Final Body Weight; DE: Digestible Energy; BWG: Body Weight Gain; FI: Feed Intake; FCR: Feed Conversion Ratio; SGR: Specific Growth Rate; PER: Protein Efficiency Ratio; Alb: Albumin

Introduction

Tilapia sometimes referred to as the "aquatic chicken" due to its rapid growth, adaptation to a broad spectrum of environmental circumstances and ability to grow and reproduce in captivity, is among the most promising species for aquaculture and has lower dietary energy requirements than other fish as it does not have to maintain a steady body temperature [1].

It has been reported that feed additives may be both nutritive and non-nutritive ingredients and work by either direct or indirect methods on the animal's system. Feed additives are supplemented in small amounts (alone or in combination) for a specific purpose, such as to improve the quality of fish as a final product, to preserve the physical and chemical quality of the diet or to maintain the quality of theaquatic environment. There are diverse ranges of feed additives used in aquatic feeds. Some dditives are used in fish feed to preserve the nutritional characteristics of a diet or feed ingredients prior to feeding (e.g. antioxidant and mold inhibitors), enhance ingredient dispersion or feed pelleting (e.g. emulsifiers, stabilizers and binders), facilitate feed ingestion and consumer acceptance of the product (e.g. feed stimulants or attractants) and promote growth (e.g. growth promoters, including antibiotics, probiotics and hormones). Also, Enzymes also used to improve the availability of certain nutrients (e.g. proteases, amylases) or to eliminate the presence of certain antinutrients (e.g. phytase) [2-4].

Guanidinoacetic acid (GAA) is available commercially as a feed additive and is more effective than arginine (Arg) or creatine (Cre) as a supplement because of its lower price and higher chemical stability [5]. GAA is a naturally occurring compound synthesis in

vivo from Arg and glycine through Arg: - glycine amidinotransferase (AGAT) or produced through chemical synthesis [6]. After formation or absorption by small intestine, GAA is converted into creatine (Cre) via guanidinoacetic methyl transferase (GAMT) and then phosphorylated to phosphocreatine (PCr), which is necessary to maintain energy homeostasis in muscle cells and act as a dynamic reservoir of high - energy phosphate (phosphocreatine/ Cre system buffering and the ATP/ADP ratio) [7,8].

The association of high-energy diets with a higher demand for muscle Cre may be due to more efficient use of dietary nutrients and energy, resulting in a particularly improved growth rate for feed conversion [9]. GAA dietary supplementation plays a central role in energy metabolism by increasing the Cre concentration and Cre phosphate: muscle ATP ratio [10]. Moreover, [11] reported that energy use could be accomplished by adding Cre or GAA as a precursor to Cre because the lack of sufficient Cre could limit the availability of methionine for protein and phosphatidylcholine synthesis, thereby limiting muscle growth. Additionally, [12] reported that GAA can compensate for Arg in Arg-deficient diets, improve growth performance and help to maintain overall energy homeostasis in bird.

Our study was conducted to clarify the relationship between GAA supplementation with varying levels of energy content and growth performance, serum metabolites, liver oxidant and antioxidant markers in Nile tilapia fish.

Materials and Methods

Experimental diets

Four experimental diets were formulated in accordance with [13] to meet the nutrient requirements of Nile tilapia fish (O. *niloticus*) (Table 1). The first experimental diet (control group, G1) contained normal levels of digestible energy (DE) for fish (3000 kcal DE/ kg diet) without any GAA supplementation. The other groups of fish (G2, G3 and G4) were fed diets with different energy levels (25, 50, and 75 kcal/kg DE less than in the control, respectively) and supplemented with GAA at a level of 0.06%. The diets were prepared in the form of a water- stable sinking pellet and stored in plastic bags in a refrigerator during the time of use.

Ingredients/kg	Dietary treatment			
	Control	-25 kcal DE	-50 kcal DE	-75 kcal DE
Yellow corn	11.99	11.89	11.89	8
Soybean meal	19	21.98	22.48	20
Fish meal	20.83	18	17.9	18.5
Corn gluten	1	1	1	1
Gelatin	1.8	1.8	1.8	1.8
Oil	1	0.63	0.2	000
Wheat bran	42.45	42.8	42.8	48.77
Min. and vit.	1	1	1	1
Salt	0.5	0.5	0.5	0.5
Vit c	0.1	0.1	0.1	0.1
Antioxidant	0.02	0.02	0.02	0.02
Dicalcium phosphate	0.1	0.1	0.1	0.1
Methionine	0.21	0.21	0.21	0.21
GAA	0.000	0.06	0.06	0.06
Calculated value				
CP%	32.36	31.94	32.1	32.01
DE	299920.03	297513.2	295257.1	292871.8
Crude fat	7.3	7.09	7.08	7.2
Crude fiber	5.3	5.39	5.42	5.84
Calcium	1.2	1.08	1.08	1.11
Phosphorus	0.57	0.54	0.54	0.56
Ash	7.5	7.14	7.15	7.4

Min. and vit.: vitamin mixture supplies the following per kilogram of diet:: vit. A -1,200.000 IU; vit. D3 - 200,000 IU; vit. E - 12,000 mg; vit. K3 - 2,400 mg; vit. B1 - 4,800 mg; vit. B2 - 4,800 mg; vit. B6 - 4,000 mg; vit. B12 - 4,800 mg; folic acid - 1,200 mg; vit. C - 48,000 mg; biotin - 48 mg; choline- 65;000 mg; niacin - 24,000 mg; Fe - 10,000 mg; Cu - 600 mg; Mg - 4,000 mg; Zn - 6,000 mg; I - 20 mg; Co - 2 mg; Se - 20 mg. CreAMINO®: a granulated product, contains a minimum of 96% chemically synthesised GAA as active substance. Glycine (≤1.5%), cyanamide (≤0.03%), dicyandiamide (≤0.5%) and melamine (≤0.002%) are impurities arising from the production process.
Table 1: Nutritional Composition of Experimental Diets

Experimental design

One hundred and sixteen monosex Nile tilapia fish weighing approximately 20 g were received and stocked in 8 glass aquaria, with 20 fish per aquarium. Each diet was fed to the fish in duplicate aquaria at 3% of body weight twice daily (8.00 h-2.00 h) for 60 days [14]. Each day the aquaria were cleaned, with partial replacement of water by previously stored (for 72 h) dechlorinated tap water. After weighing the fish, the dietary allowances of each experimental group were adjusted bi-weekly, according to mean body weight and then placed in small plastic bags for each aquarium for the daily feeding of fish.

Growth performance indices

Fish were observed daily to monitor any changes in growth or water quality and exclude any dead fish. The following indices of growth and feed utilization were calculated at the end of the experimental period (body weight gain (BWG), feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER).

BWG (g) = Mean FBW (g) - Mean initial BW
(FCR) = Total dry weight of feed / BWG
SGR (%/day) = 100 x [(Ln (FBW) - Ln (mean initial BW)]/culture period (days)
PER =Wet weight gain/crude protein fed (g).

Sample collection

At the end of the experimental period (60 days), blood samples were collected from the caudal vein of 9 fish/ treatment in a plain tube , kept at room temperature for 20 min to allow clotting, and then stored in a refrigerator for 4 h. The clear serum was then carefully separated by centrifugation at 3000 rpm for 10 min and stored at -20 °C for the estimation of selected biochemical parameters. One gram of liver was rapidly collected from each fish and then washed in ice-cold saline buffer (20 mM Tris–HCl, 0.14 M NaCl buffer, pH 7.4), followed by homogenization in ice cold phosphate buffered saline (PBS) (pH 7.4). The liver homogenate was centrifuged for 15 min at 3000 rpm and at 4 °C and the supernatants were then collected carefully and stored at -80 °C for the estimation of oxidative stress and antioxidant biomarkers [15].

Blood biochemical analysis

Serum samples were analysed spectrophotometrically (BM Co., Germany, 5010) to estimate their Cre kinase (CK) levels by using a commercial kit provided by ELI Tech Company according to [16]. Serum total protein (TP) and albumin (Alb) were evaluated using Stanbio Laboratory USA kits according to [17]. Serum globulin (Glob) concentration was calculated by subtracting Alb from TP after which the Alb to Glob ratio (A/G ratio) was calculated according to [18]. Creatinine (Cr) and urea were assayed by using ready-made kits manufactured by Human Company Germany and Diamond kits according to [19,20] respectively. Cholesterol, triglycerides and high density lipoprotein HDL cholesterol were assessed according to [21] using kits produced by Spinreact Spain.

Liver oxidative markers and antioxidant capacity

Lipid peroxidation (malonaldehyde (MDA) and antioxidant markers, and reduced glutathione (GSH), superoxide dismutase (SOD), and nitric oxide (NO) in liver homogenate samples were spectrophotometrically determined by the enzymatic colorimetric method using commercially available kits (Bio-diagnostic, Egypt).

Statistical analysis

Statistical analysis was carried out using SPSS 20 to test the impact of GAA supplementation of fish diets with varying energy contents. One-way ANOVA and Duncan's multiple comparison test were applied to compare means and standard errors. Differences between treatments were considered significant when $P < 0.05$.

Results

Growth performance indicators

The impacts of GAA supplementation with different energy contents on the growth performance parameters of Nile tilapia fish are presented in Table 2. Fish in G2 and G3 had significantly ($P < 0.05$) higher body weights (55.48 g ± 2.08 and 58.74 g ± 3.65, respectively) than those in G1 and G4 were (51.55g±1.48 and 52.40±1.95) respectively.

Fish in G4 had significantly ($P < 0.05$) the lower BWGS (32.90 g ± 0.62) than those in the other experimental groups. The highest BWG was recorded in G2, followed by G3 and G1 (39.44 ± 1.3, 38.44 ± 1.56 and 37.58 ± 1.62, respectively). There were no significant differences in the FCR among G1, G2, and G3. Moreover, the highest FCR and the lowest PER were recorded in G4. The highest SGR was recorded in G3.

Parameters	Control	-25 kcal DE	-50 Kcal DE	-75 kcal DE
Initial weight (g)	21.20±1.78	21.53±1.23	21.20±1.53	21.25±1.57
Final weight (g)	51.55±1.48^{b}	55.48±2.08^{a}	58.74±3.65^{a}	52.40±1.95^{b}
Body weight gain (g)	37.58±1.62^{a}	39.44±1.30^{a}	38.44±1.56^{a}	32.90±0.62^{b}
FCR	2.13±0.055^{b}	2.23±0.067^{b}	2.14±0.083^{b}	2.65±0.051^{a}
SGR	1.47±0.098^{b}	1.47±0.080^{b}	1.78±0.16^{a}	1.49±0.097^{b}
PER	1.45±0.037^{a}	1.39±0.041^{a}	1.43±0.045^{a}	1.17±0.022^{b}

^{abc} Means in the same row with the different superscript letter are significantly difference ($P < 0.05$)

Table 2: Effect of GAA Supplementation with Reduction of Energy Level on Growth Performance of Nile Tilapia Fish

Blood biochemical analysis

GAA supplementation with different levels of energy content did not have any significant effects on serum Alb, TP or Glob levels ($P < 0.05$) or the A/G ratio. However, the blood serum levels of glucose and urea in G4 were significantly higher than those in the other groups. Moreover, the serum cholesterol and triglyceride levels ($p>0.05$) in G2, G3 and G4 were significantly higher than those in G1. GAA supplementation in G2 and G3 led to higher serum Cr level than in other groups (Table 3).

Parameter	Control	-25 energy	-50 energy	-75 energy
CK	4.97±0.79^{c}	14.80±2.52^{c}	54.56±8.29^{b}	72.57±3.52^{a}
T.P	4.15±0.38	4.44±0.52	4.75±0.81	3.92±0.50
Albumin	2.42±0.18	2.56±0.12	2.88±0.66	2.57±0.24
Globulin	1.72±0.30	1.88±0.64	1.86±0.40	1.35±0.32
A/G ratio	1.50±0.26	0.67±0.30	1.71±0.48	1.83±0.54
Glucose	13.49±2.27^{c}	18.02±6.09^{c}	39.07±2.56^{b}	49.41±1.81^{a}
T.G	235.56±5.69^{b}	270.81±7.86^{ab}	281.33±25.95^{ab}	291.00±15.27^{a}
Chol	137.21±8.65^{c}	186.02±7.25^{ba}	220.03±11.75^{a}	200.54±6.25^{a}
Urea	14.76±1.69^{b}	17.84±2.99^{b}	25.16±2.01^{b}	44.51±6.18^{a}
Creatinine	1.38±0.19^{b}	1.60±0.15^{ab}	1.61±0.02^{ab}	1.26±0.01^{b}

^{abc} Means in the same row with the different superscript letter are significantly difference ($P < 0.05$)

Table 3: Influence of GAA Supplementation and Different Energy Levels on Serum Metabolites of Nile Tilapia Fish

Liver oxidative and antioxidant markers

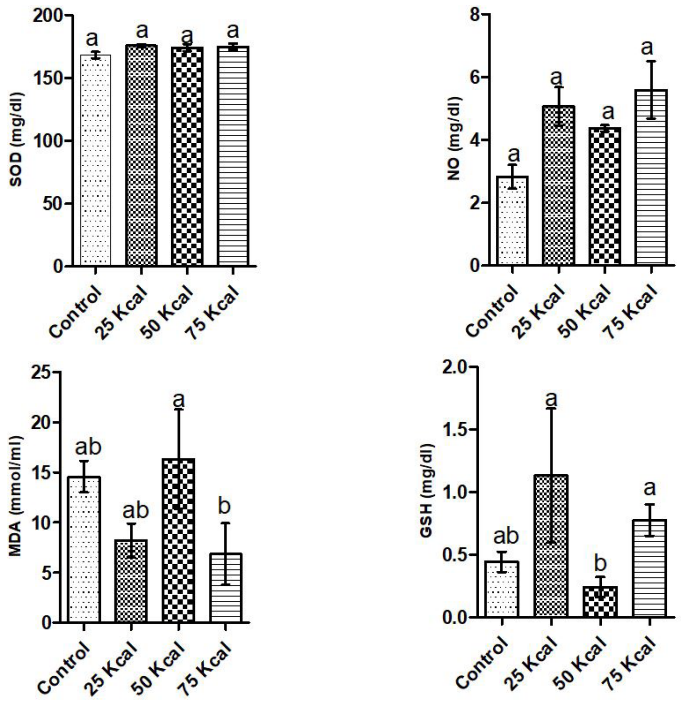

Figure 1: Serum oxidative and antioxidant markers under supplementation with 0.06% GAA and different energy levels (- 25, - 50, and - 75 kcal/kg). P value < 0.05

Supplementation of fish diets with GAA and a reduction in energy content of -75 kcal resulted in a significant decrease in tissue MDA level compared to the other experimental treatments. Non-significant differences in tissue SOD & NO levels were observed in all groups. Moreover, the tissue GSH levels were significantly higher ($P < 0.05$) in G1, G2 and G4 than in G3 Figure 1.

Discussion

Our results revealed that 0.06% GAA supplementation of fish diets combined with an energy content of up to 50 kcal/ kg DE less than that in the control was able to improve the growth performance of G2 and G3 fish in the form of FBW and BWG. Similarly, [22] researched the impact of GAA supplementation of the diets of broiler chickens with different energy contents and concluded that dietary supplementation with 1.2 g / kg GAA elevated the growth and improved the FCR of broiler chickens fed low metabolizable energy (ME) diets. Moreover, beneficial effects of GAA were more prominent in low energy diets. Additionally, [23] studied the effect of different levels of energy reduction compared to a control and supplementation with GAA on broiler chickens and found no significant differences in the BWG of poultry during the grower period due to an interaction between the level of energy and supplementation with CreAM (as a source of GAA). Moreover, broiler receiving 25 kcal/kg ME less energy or control level energy with CreAM supplementation gained more weight than those supplemented with CreAM and low energy 75 kcal / kg. In contrast, [24] found that the interactions between ME levels in poultry and GAA supplementation did not significantly affect BWG. Our results are similar to the results obtained by [25] who examined the impact of adding GAA to broiler diets with variable energy contents and observed a greater response to GAA at higher AMEn levels. In addition, the growth rate improved with decreasing AMEn level and reached a peak at an AMEn content of approximately 97%. Our results regarding the effects of the relationship between GAA and different levels of energy on FBW and BWG revealed a greater reduction in the effect of GAA at higher energy levels. The Positive effect of GAA on growth performance may be ascribed to its main role as a Cre precursor and its ability to increase muscular Cre and ATP stores [26]. Furthermore, GAA contributes to Cre formation and conserves Arg, which can then be used by the body for other functions such as protein anabolism [27].

The FCR is a more sensitive indicator of nutrition adequacy than the growth rate. The data revealed no significant differences in the FCR among G1, G2 and G3, and the highest FCR (2.65) was found for fish in G4. The present findings are in agreement with those of [9], who showed lower FCR at higher energy levels and with GAA supplementation, resulting in lower caloric intake per kilogram of BWG resulting from an improved FCR. Similar to our results, [23] found that the energy level and CreAM supplementation affected the FCR and that the addition of CreAM to reduced-energy diets led to an enhanced FCR. Furthermore chickens that received the control energy level had the best FCR, followed by those that received 50, 25 and 75 kcal/kg less ME. However, [24] found that the effect of GAA on the FCR decreased with lower levels of AMEn in poultry diets. Moreover, [27] found that fish fed diets containing 2,800 kcal/kg DE had lower FCR than those fed the other diets containing different DE levels (2600 and 3000). The data from our study showed that the SGR and PER of Nile tilapia fish in G3 were significantly higher than those in fish in the other experimental groups. This result may be attributed to GAA acting as a commercially a viable compound to progress growth performance by sparing Arg for use in protein synthesis [28].

The Findings showed that creatine kinase (CK) levels in the serum of fish in G4 were significantly higher than those in fish in the other experimental groups. [12] indicated that CK is extremely abundant in tissues with high and fluctuating energy demand and help in the transformation of high-energy phosphates between ATP and phosphocreatine. The modification of CK activity represents the muscle energy storage and conversion status. GAA can promote the development of Cre and increase CK activity [29]. Increased levels of circulating CK indicate a potential role of GAA in maintaining high ATP turnover at low temperatures [30]. Using different levels of GAA did not significantly influence the CK values in newly weaned piglets at 63 days of age [26].

In our study, there were no significant effects ($P > 0.05$) of the experimental diets on the variables of serum biochemistry analysed (TP, Alb, Glo and A/ G ratio). Also, [31] found that serum protein, Alb, cholesterol and glucose remained constant ($P > 0.05$), even if the amount of GAA incorporated in the diet was as high as 0.6%.

These findings are in contrast to those of [22] who reported that the interaction between GAA supplementation and dietary energy level of improved the serum concentration of TP. In addition, the serum level of TP in chickens fed a low - energy diet supplemented with 1.2 g/kg GAA was greater than that in chickens fed a control diet, which further supports the idea that GAA supplementation enhances the use of protein in broiler chickens, particularly when their dietary energy is restricted.

GAA supplementation of Nile tilapia fish with a reduction in dietary energy level of 25 or 50 kcal DE led to a higher serum Cr level than in other groups. Similarly, [22] found that adding 1.2 g / kg of GAA to broiler diets with a decreased energy content led to greater serum Cr concentration compared that in broilers fed standard basal diets. Similarly, [26] noted that the level of Cr in the blood of newly weaned piglets was not substantially affected by the addition of GAA to the diet. GAA supplementation and dietary energy amount influenced the concentrations of serum cholesterol and triglyceride ($P < 0.05$) in all experimental groups compared to the control group. There was a significant ($P < 0.05$) increase in serum glucose level in the G4 compared with other experimental groups. Similarly, [32] observed that GAA as a Cre precursor can directly increase the ATP and phosphocreatine contents and glucose decomposition to generate energy. There was a significant ($P < 0.05$) decrease in tissue MDA levels in G4 compared to the other experimental groups. Similarly, [33] showed that Cherry Valley ducks fed diets supplemented by GAA exhibited lower

plasma concentrations of MDA than ducks fed a control diet. In addition, the concentration of MDA in growing pigs fed GAA containing diets was reportedly lower than that in growing pigs fed a control diet [34]. However, [35] postulated that the serum MDA concentration tended to improve with the addition 2 g / kg GAA. In our study, the level of reduced glutathione in G1 did not differ significantly from that in G2 or G4, and the highest level of reduced glutathione was found in G2.

Our results are consistent with the results obtained by [35] who concluded that GAA has an oxidant - antioxidant capacity. In addition, GAA improves the total antioxidant capacity and the activity of multiple antioxidant enzymes.

Conclusion

In conclusion, the reduction of DE by up to 50kcal/kg combined withsupplementation with GAA at a level of 0.06% improved the growth performance of Nile tilapia fish.

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