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Original Research Articles

Determination of Growth and Nutritional Composition of Nile Tilapia (*Oreochromis niloticus*) Fed With L-Glutamic Acid Supplemented Feeds

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The study aimed to assess Nile tilapia's (*Oreochromis niloticus*) growth performance and body chemical composition through varying levels of L-Glutamic acid supplementation. In the experiment, four different groups of feed were prepared; the control C (0% L-Glutamic acid), G1 (1% L-Glutamic acid), G2 (2% L-Glutamic acid), and G3 (3% L-Glutamic acid). Tilapia juveniles with an average initial weight of 4.86 g were stocked into 12 fiberglass tanks (450L) with 40 individuals and three replications under controlled conditions, and the experiment was continued for a period of 60 days. At the end of the experiment, for the G2 group final weight (FW, 19.31±0.59 g), specific growth rate (SGR; 2.30±0.05), feed conversion ratio (FCR; 1.02±0.03), daily growth rate (DGR; 4.95±0.20) protein efficiency ratio (PER 2.83±0.10) and net protein utilization (NPU; 61.62±3.39) were better than the other groups ($P<0.05$). Nutritional composition data showed that the groups differed between protein, dry matter, and lipid compositions. The G2 group exhibited the highest whole-body protein level, recording a value of 21.24±0.52, whereas the control group demonstrated the lowest protein level at 20.17±0.15. In conclusion, incorporating 2% L-Glutamic acid into the diet of juvenile Nile tilapia is advisable for both the growth and development of the fish and for enhancing their nutritional composition.

INTRODUCTION

The Tilapia species belonging to the Cichlidae family is one of the most extensively cultivated fish worldwide. Its fresh and processed products are consumed in various regions across the globe. Tilapia farming is practiced in certain tropical and subtropical countries through extensive or semi-intensive cultivation methods.[1] The global tilapia sector has exhibited rapid growth trends. Despite the challenges posed by the COVID-19 pandemic, the total global tilapia production reached 6 million tons for the first time in 2020, showing a 3.3% increase.[2] Tilapia production is increasing every year worldwide. There has also been an interest in tilapia production in Turkey, and a commercial company in Konya started producing red tilapia (*Oreochromis sp.)* in 2014.[3] In 2020, the total production of Nile tilapia was recorded at 4,407,200 tons. Notably, the global production of Nile tilapia has quadrupled from 1,101,500 tons in the year 2000 to the observed levels in 2020.[4]

It is well known that the feeding activity of fish is closely related to the senses of sight and taste. Fish possess highly developed taste receptors that are crucial to their feeding patterns (Goh and Tamura). Various factors influence the preference of fish for certain feeds. These factors primarily encompass the feed's appearance, scent, and taste. Generally, enticing substances in the feed elicit a positive effect that drives the fish to approach, bite, taste, and eventually consume the feed.[5]

Consequently, the utilization of attractants in feeds, whether natural or synthetic, has been on the rise in recent years. Attractants typically possess a low molecular weight and contain nitrogen or nitrogen-containing structures. They are water-soluble compounds that are both acidic and basic and exhibit amphoteric properties. These additives, known as feed attractants, enhance or stimulate cultured aquatic organisms' feed consumption. They can be in various forms, the most notable being free amino acids and nucleotides.[6] The significance of certain chemical stimulants in fish nutrition has been acknowledged, including the chemical stimulatory effects of L-amino acids, nucleotides, and nucleosides.[7]

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Betaine and amino acids can readily dissolve in water, and these substances within the feed can quickly disperse into the water upon contact.[8] L-alanine, L-glutamic acid, L-arginine, glycine, betaine, inosine, and inosine-5-phosphate can serve as attractants in certain species.[9] Initial studies in this field have demonstrated the potential utility of different attractants for various fish species.[10-14]

The feeding habits of fish play a role in the selection of attractants, exhibiting distinct differences.[5] For carnivorous and omnivorous species, betaine, amino acids (especially glycine and alanine or L-amino acid mixtures), nucleotides, inosine, or inosine 5-monophosphate mixtures have been reported to be stimulatory.[15-17] In herbivorous species, data are relatively limited; however, certain amino acids and dimethyl-beta-propiothetin have been found to stimulate carp and tilapia in conjunction with organic acids. Among carnivorous species, alkaline and neutral additives such as glycine, proline, taurine, valine, and betaine are preferred. On the other hand, herbivorous and omnivorous species tend to favor more acidic compounds like aspartic and glutamic acids.[16,18]

L-glutamic acid dissolves and diffuses in water, subsequently entering the citric acid cycle and being utilized by fish for energy purposes.[8,14] It is known that certain L-amino acid mixtures stimulate feed intake in species like rainbow trout (*Oncorhynchus mykiss*),[10] European sea bass (*Dicentrarchus labrax*),[15] and European eels (*Anguilla anguilla*).[11] Compounds like glutamic acid, aspartic acid, lysine, citric acid, and malic acid have enhanced nutrient intake in *Tilapia zillii*.[12]

According to the literature, specific studies evaluating the effects of L-glutamic acid on Nile tilapia have not been reported. Therefore, the objective of the present study was to determine the effect of different levels of dietary L-glutamic acid on the growth, feed utilization, and nutritional composition of Nile tilapia fingerlings. Thus, our research holds considerable importance in tilapia farming.

MATERIALS AND METHODS

EXPERIMENTAL MATERIALS

In the trial, *Oreochromis niloticus* (Nile tilapia) individuals were obtained from the Çukurova University Faculty of Fisheries, Dr. Nazmi TEKELİOĞLU Research Station, with an average weight of 3-5 grams. These fish were then held in stock tanks for approximately two weeks for acclimatization. During acclimatization, the fish were fed three times a day with a control group diet. The composition of the diets used in our research is provided in Table 1. All the raw materials required to formulate the diets were sourced from local commercial suppliers. The diets had uniform crude protein content (35% CP; iso-proteic) and crude lipid content (12% CL; iso-lipidic) (**Table 1**).

EXPERIMENTAL DESIGN

The study was conducted in the Dr. Nazmi TEKELİOĞLU Research Station at Çukurova University, Faculty of Fisheries. During measurements, Tilapia individuals with an average initial weight of 4.86±0.62 g were used and anesthetized with the anesthetic substance (phenoxyethanol; Sigma, St. Loui(s, MO to ensure they were not harmed. After total length and weight measurements, the fish were stocked in 450 L volume fiberglass tanks, with 25 individuals each, and in triplicate. The groups to be tested in the trial were arranged as follows: Control (C) (0% L-Glutamic acid), Group 1 (G1): (1% L-Glutamic acid), Group 2 (G2): (2% L-Glutamic acid), Group 3 (G3): (3% L-Glutamic acid).

EXPERIMENTAL METHODS

The formulas of the growth parameters used in the research are given below;

IW: Initial Weight (g), FW: Final Weight (g),

Daily Growth Rate (DGR; %/day) = ((Weight Gain %)/days),[18]

Specific Growth Rate (SGR) = (ln Final Weight – ln Initial Weight) / days ×100,[19]

Thermal Growth Coefficient (TGC) = ((Final Weight $^{1/3}$–Initial Weight$^{1/3}$)/average temp.*days)*1000,[18]

Condition Factor (CF) = 100*(Fish Weight (g))/(Fish Length (cm))^{3}.[18]

The formulas of the feed utilization parameters used in the research are given below;

Feed Conversion rate (FCR)= Total dry feed consumed (g) / Weight Gain (g),[20]

Lipid Efficiency Ratio (LER)= Weight Gain (g) / mass of lipid fed (g),[18]

Protein Efficiency Ratio (PER)= Weight Gain (g) / mass of protein fed (g),[21]

Net Protein Utilization (NPU)= [(Final body protein (g) – Initial body protein (g))/protein intake (g)]×100,[18]

Net Lipid Utilization (NLU)= [(Final body lipid (g) – Initial body lipid (g))/lipid intake (g)]×100,[18]

Hepatosomatic Index (HSI)=100× [Liver weight (g)/Body weight(g)],[22]

Viserosomatic Index (VSI)= 100× [Visceral weight (g)/Body weight(g)].[22]

PROXIMATE ANALYSIS

The proximate composition of the fish samples was analyzed in triplicates using the following methods. The lipid content was determined using the method proposed by Bligh and Dyer.[23] The fish's moisture, protein, and ash content were measured using the AOAC (Association of Official Analytical Chemists) method.[24]

STATISTICAL ANALYSIS

The statistical analysis assessed the differences between the data obtained from the control and experimental groups. The results are presented as means ± standard error. One-way analysis of variance (ANOVA) and Duncan's post-hoc test[25] was performed using the SPSS 21 software (SPSS) to determine the statistical significance of these differences. P-values less than 0.05 were considered to be statistically significant.

Table 1. Formulation and proximate composition of experimental diets

	EXPERIMENTAL GROUPS			
Ingredients (g/kg)	**C**	**G1**	**G2**	**G3**
Fish meal	220	220	220	220
Corn Gluten	160	150	140	130
Wheat bran	360	360	360	360
Fish oil	35	35	35	35
Sunflower oil	35	35	35	35
Carboxy Methyl Cellulose (CMC)	80	79.9	79.8	79.7
Dicalcium Phosphate (DCP)	65	65	65	65
Vitamin mix[1]	25	25	25	25
Mineral mix[2]	15	15	15	15
L-Lysine	3	3	3	3
DL-Methionine	2	2	2	2
L-Glutamic acid	0	10.1	20.2	30.3
Total	**1000**	**1000**	**1000**	**1000**
Chemical composition (dry matter %)				
Moisture	0.93	0.93	0.93	0.93
Crude protein	34.49	34.47	34.46	34.45
Crude lipid	12.73	12.66	12.58	12.51
Ash	13.61	13.57	13.54	13.50
Nitrogen Free Extract (NFE)[3]	40.51	40.53	40.55	40.57
Gross Energy (GE)[4] (MJ/kg)	20.03	20.00	19.97	19.94
P:E[5]	17.22	17.24	17.26	17.27

[1]Vitamin mix: kg/feed 4 000 000 IU vit. A, 480 000 IU vit. D3, 40 000 mg vit. E, 2400 mg vitamin K3, 4 000 mg vitamin B1, 6 000 mg vitamin B2, 40 000 mg niacin, 10 000 mg calcium D-pantotenat, 4 000 mg vitamin B6, 10 mg vitamin B12, 100 mg D-biotin, 1 200 mg folic acid, 40 000 mg vitamin C ve 60 000 mg inositol

[2] Mineral mix: kg/feed 23 750 mg Mn, 75 000 mg Zn. 5 000 mg Zn, 2 000 mg Co, 2750 mg I, 100 mg Se, 200 000 mg Mg

[3] NFE (Nitrogen Free Extract): 100 - (Crude Protein + Crude Lipid + Ash + Fiber).

[4] Gross Energy (GE) (MJ/kg): 23.4 MJ/kg protein, 39.2 MJ/kg lipid and 17.2 MJ/kg NFE.

[5] P:E = Protein / Energy

RESULTS

During the experimentation, the temperature of the water fluctuated between a minimum of 26°C and a maximum of 28°C, with an average of 27 ± 0.50°C. The highest and lowest dissolved oxygen (O_2) values recorded were 7.5 mg/L and 6.5 mg/L, respectively.

According to the results obtained at the end of the study, significant differences were observed in the final weight values among the groups ($p<0.05$). The highest final weight was found in the G2 group (19.31±0.56), followed by the G1 group (18.69±1.09). The other two groups had the lowest final weight. Specific growth rates also exhibited significant differences among the groups ($p<0.05$). The highest specific growth rate was observed in the G2 group (2.30±0.05), followed by the G1 (2.24±0.10), C (2.17±0.08), and G3 (2.13±0.09) groups, respectively. In the study, there was a statistically significant difference in the daily growth rate among the groups ($p<0.05$). The highest DGR was 4.95±0.20 in G2, while the lowest value was 4.32±0.31 in G3. The thermal growth coefficient (TGC %/day) value differed among the groups ($p<0.05$) as well. The highest TGC was found in G2 (0.61±0.02), with the other experimental groups trailing behind. Condition factor (CF) was expressed as a height-weight index and was found to be different between groups ($p<0.05$). The highest CF was found in the G2 group with 1.57±0.16 and the lowest in the G3 group with 1.50±0.12. All growth parameters are given in **Table 2**.

The feed conversion ratio (FCR) showed statistical differences among the groups ($p<0.05$). The highest FCR result was observed in the G3 group (1.20±0.08), followed by the C group (1.13±0.09), while the other two groups exhibited lower values ranging between 1.02 (G2) and 1.06 (G1). Protein efficiency ratio (PER) values displayed significant differences among the groups as well, with the highest value of 2.83±0.1 found in the G2 group, and the lowest value of 2.43±0.17 observed in the G3 group ($p<0.05$). Net protein utilization (NPU) values also showed statistical differences ($p<0.05$), with the highest NPU value of 61.62±3.39 in the G2 group, followed by G1 (58.71±5.62), C (53.33±4.75), and G3 (51.54±2.86) groups, respectively. Lipid efficiency ratio (LER) exhibited the lowest values in the C (7.73±0.65) and G3 (7.30±0.51) groups, while the highest value was observed in the G2 group (8.50±0.31), followed by the G1 group (8.22±0.54) ($p<0.05$). The highest net lipid utilization (NLU) value was determined in the G2 (27.46±2.05) and G1 (27.07±2.42) groups, followed by the C group (24.77±2.75). The NLU value for the G3 group was found to be the lowest

Table 2. Growth parameters in experimental groups

	EXPERIMENTAL GROUPS			
	C	G1	G2	G3
IW	4.86±0.04	4.86±0.07	4.86±0.04	4.86±0.02
FW	17.84±0.79^{b}	18.69±1.09^{ab}	19.31±0.59^{a}	17.46±0.93^{b}
SGR	2.17±0.08^{bc}	2.24±0.10^{b}	2.30±0.05^{a}	2.13±0.09^{c}
DGR	4.45±0.27^{c}	4.74±0.37^{b}	4.95±0.20^{a}	4.32±0.31^{d}
CF	1.53±0.09^{b}	1.54±0.09^{b}	1.57±0.16^{a}	1.50±0.12^{c}
TGC	0.57±0.02^{b}	0.59±0.03^{ab}	0.61±0.02^{a}	0.56±0.03^{b}

Table 3. Feed evaluation parameters in experimental groups

	EXPERIMENTAL GROUPS			
	C	G1	G2	G3
FCR	1.13±0.09^{ab}	1.06±0.07^{b}	1.02±0.03^{b}	1.20±0.08^{a}
PER	2.58±0.22^{c}	2.74±0.11^{b}	2.83±0.10^{a}	2.43±0.17^{d}
LER	7.73±0.65^{c}	8.22±0.54^{b}	8.50±0.31^{a}	7.30±0.51^{c}
NPU	53.33±4.75^{c}	58.71±5.62^{b}	61.62±3.39^{a}	51.54±2.86^{d}
NLU	24.77±2.75^{b}	27.07±2.42^{a}	27.46±2.05^{a}	22.58±1.04^{c}

at 22.58±1.04 ($p<0.05$). The feed evaluation parameters obtained at the end of the study are given in **Table 3**.

At the beginning of the study, data obtained from initial sampling indicated the following composition percentages; dry matter content was found to be 24.03±0.74, ash content was 3.12±0.13, protein content was 18.75±0.10, and lipid content was 2.89±0.07. Initially, HSI (Hepatosomatic Index) and VSI (Viscerosomatic Index) values were determined to be 1.92±0.05 and 2.15±0.08, respectively.

At the end of the research, differences were observed in the protein compositions of the whole body among the groups ($p<0.05$). The highest protein content was found in the G2 group (21.24±0.52) ($p<0.05$), followed by C (20.17±0.15), G1 (20.73±0.98), and G3 (20.51±0.22) groups, respectively.

Similarly, lipid composition also varied among the groups ($p<0.05$). The highest lipid values were observed in the G1 (3.19±0.07), G2 (3.14±0.08), and C (3.12±0.06) groups. The lowest lipid content was determined in the G3 group (3.04±0.05).

When examining the percentage of total body dry matter, statistically significant differences were observed among the groups ($p<0.05$). The highest dry matter content was observed in the G2 (26.90±0.15) and G3 (26.64±0.41) groups, while other group individuals closely followed with similar values.

For ash content, statistical differences were also found ($p<0.05$). The highest ash content was observed in the G2 group with a value of 3.35±0.07, and in the G3 group with a value of 3.43±0.85. There was no statistical difference between the other two experimental groups.

Regarding the Hepatosomatic Index (HSI) and Viscerosomatic Index (VSI), statistical differences were found among the groups ($p<0.05$). When examining the data, it was determined that the highest HSI value was 3.13±0.75 in the G3 group, and the lowest HSI value was 2.99±0.46 in the G1 group. VSI values were highest in the G1 group (3.92±0.56), while the lowest value was observed in the G3 group (3.72±0.74) ($p<0.05$). The fish whole body proximate composition analysis results, HSI, and VSI values are provided in **Table 4**.

DISCUSSION

In this study, juvenile Nile tilapia was subjected to varying doses of L-glutamic acid as an attractant additive in their feeds, and the effects were assessed.

Water temperature is a critical factor influencing fish feed intake and growth. Therefore, it is inevitable that appetite disturbances and consequent weight loss occur at low or high-water temperatures when optimal conditions are not met.[26,27] Our research has been carried out under conditions where optimal water parameters were maintained.

Evidently, growth parameters (FW, SGR, DGR, TGC, and CF) in the G2 group were better than in other groups. The obtained outcomes align with previous studies of different fish species and shrimp. These are the stimulating effects of L-amino acid mixtures for European eels (*Anguilla anguilla*),[11] the capacity of glutamic acid, aspartic acid, lysine, citric acid, and malic acid to enhance food intake in *Tilapia zillii*,[12] the significance of L-proline and L-glutamic acid as primary taste stimulants for black rabbitfish (*Siganus fuscescens*),[28] and the improvement in live weight gain of juvenile shrimp (*Penaeus monodon*) through betaine/amino acid blends.[29]

It is important to include attractant additives in feed that can stimulate growth, development, feed intake and

Table 4. Proximate composition and somatic indexes in experimental groups

	EXPERIMENTAL GROUPS				
	Initial	**C**	**G1**	**G2**	**G3**
Protein	18.75±0.10	20.17±0.15^{b}	20.73±0.98^{b}	21.24±0.52^{a}	20.51±0.22^{b}
Lipid	2.89±0.07	3.12±0.06^{a}	3.19±0.07^{a}	3.14±0.08^{a}	3.04±0.05^{b}
Dry matter	24.03±0.74	25.35±0.45^{b}	25.70±1.21^{b}	26.90±0.15^{a}	26.64±0.41^{a}
Ash	3.12±0.13	3.09±0.11^{b}	3.05±0.32^{b}	3.35±0.07^{a}	3.43±0.85^{a}
HSI	1.92±0.05	3.09±0.54^{ab}	2.99±0.46^{c}	3.05±0.60^{b}	3.13±0.75^{a}
VSI	2.15±0.08	3.86±1.08^{b}	3.92±0.56^{a}	3.85±0.85^{b}	3.72±0.74^{c}

appetite. Determining the appropriate dosage is also very important in this study and similar studies.

Another noteworthy aspect of our study is that the utilization and the efficiency of protein and lipid in the groups did not correlate positively with increasing doses of L-glutamic acid. In other words, they showed values nearly identical to the control group. Similarly, the FCR (Feed Conversion Ratio) value significantly increased in the 3% L-glutamic acid group. Tekelioğlu et al.[14] conducted a study on sea bass by adding Glutamic acid and DL-Alanine at 1% and 2% levels, respectively. The best results were found in the groups with 1% additions, while the 2% level groups showed similarities to the control group.

Additionally, Shankar et al.[30] experimented with four different betaine levels (0, 0.25, 0.50, 0.75) in the feeds of fingerling Indian carp. In the study, the best growth parameters were observed in the group with 0.25% betaine addition, while the other groups remained at lower levels compared to this group. The findings of these studies, similar to our research, indicate that attractant additives beyond a certain threshold do not exhibit a positive effect. Therefore, it is evident that determining threshold values, especially in terms of growth and development parameters, is crucial for establishing appropriate dosages.

On the other hand, in the trial conducted by Zhao et al.[31] with Jian carp, different dosage levels (0, 4, 8, 16, 32 g/kg) of L-glutamate were used. It was observed that the group with the best final weight was the one with 32 g/kg supplementation, while the groups with lower dosage levels did not exhibit the same level of growth. In this context, not only determining the dosages but also identifying the specific attractant additive used and which fish species it is employed for, as well as the life stage at which it is applied, becomes crucial. As mentioned earlier, it is well-established that acidic compounds such as L-glutamic acid stimulate food intake in tilapia species and, consequently, contribute positively to growth.[12]

While the FCR values obtained at the first two dosage levels (G1 and G2 groups) appear statistically similar, it is evident that the G2 group yielded better results. Conversely, the G3 group, representing the other dosage level, exhibited the lowest FCR ratio. This observation is consistent with the growth parameters. The results indicate that fish do not utilize feeds with high levels of attractant substances effectively, highlighting the presence of a dosage threshold. Therefore, as in our study, similar research employing different dosage levels also reveals breakpoints.[14, 30-33]

It is observed that the whole-body protein composition increased compared to the initial values, and concurrently, it was at a higher level in the G2 group compared to the other groups. Additionally, at the end of the study, body lipid levels showed similarity across all groups. This suggests that the fish utilized the ingested protein for storage, thereby contributing to meat quality while efficiently utilizing the dietary lipid for energy purposes.[34] These findings are consistent with research indicating effective lipid utilization by fish and a high body-protein ratio.[31,35-37]

In our study, small differences were observed in the HSI values obtained. The HSI value of Group G2 demonstrates that the fish were healthy and efficiently utilized the feed. Additionally, when the visceral somatic index (VSI) value is considered in conjunction with the condition factor (CF) value, it indicates a parallel in growth and effective feed utilization. Consistent with findings from previous research, the HSI, VSI, and CF values obtained in Group G2 suggest that the fish effectively intake the feed, obtaining maximum benefits from protein and lipids, consequently positively influencing growth.[31,35,38]

As a result, under optimal aquaculture conditions, the addition of L-Glutamic acid at varying doses to feeds containing 35% crude protein (CP) and 12% crude lipid (CL) had no adverse effects on growth, feed utilization, and whole-body nutritional composition in Nile tilapia. Furthermore, the inclusion of 2% L-Glutamic acid is recommended based on the positive outcomes observed in all parameters.

INTEREST OF CONFLICT

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

Methodology: Yılmaz Dağdelen (Equal), Oğuz Taşbozan (Equal). Formal Analysis: Yılmaz Dağdelen (Equal), Oğuz Taşbozan (Equal). Investigation: Yılmaz Dağdelen (Equal), Oğuz Taşbozan (Equal). Writing – original draft: Yılmaz Dağdelen (Equal), Oğuz Taşbozan (Equal). Conceptualization: Oğuz Taşbozan (Lead). Writing – review & editing:

Oğuz Taşbozan (Lead). Funding acquisition: Oğuz Taşbozan (Lead). Supervision: Oğuz Taşbozan (Lead).

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The EU Directive 2010/63/EU's guidelines for animal welfare and experimental ethics were followed in this investigation. In addition, this study received ethical approval from the Animal Experiments Local Ethics Committee of Çukurova University with decision number 9, on 10.12.2020.

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