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Citric Acid-Induced Modulation Of Protein Metabolism And Nucleic Acid Dynamics In Channa Striatus: Insights Into Metabolic Reprogramming And Cellular Biosynthesis

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Abstract

Dietary organic acids significantly influence fish metabolism through complex biochemical pathways affecting protein synthesis, amino acid metabolism, and nucleic acid dynamics. This study investigated the temporal effects of graded citric acid supplementation (2%, 4%, and 6%) on tissue-specific protein content, free amino acid profiles, nucleic acid concentrations, and blood glucose levels in Channa striatus over 28 days. Fish were fed experimental diets and sampled at 7, 14, 21, and 28 days for biochemical analysis of muscle and liver tissues. Protein quantification utilized Bradford assay, amino acids were analyzed by ninhydrin method, and nucleic acids were extracted and quantified spectrophotometrically. Results revealed significant dose-dependent increases in tissue protein content, with 6% citric acid treatment achieving 84.86% and 49.14% increases in muscle and liver proteins, respectively, after 28 days. Conversely, free amino acid levels decreased progressively, indicating enhanced incorporation into protein synthesis pathways. RNA content increased substantially in both tissues (23.05% in muscle, 40.05% in liver with 6% treatment), while DNA showed modest but significant increases at higher doses. Blood glucose levels decreased significantly across all treatments, suggesting improved glucose utilization efficiency. These findings demonstrate that citric acid supplementation orchestrates a comprehensive metabolic reprogramming characterized by enhanced protein anabolism, accelerated

nucleic acid synthesis, and optimized glucose metabolism, providing mechanistic insights into growth enhancement in aquaculture species.

Keywords: Protein metabolism, nucleic acid synthesis, amino acid catabolism, glucose homeostasis, metabolic reprogramming

1. Introduction

The optimization of fish metabolism through nutritional interventions represents a cornerstone of modern aquaculture science, with particular emphasis on understanding the biochemical mechanisms underlying growth enhancement and metabolic efficiency. Protein metabolism, nucleic acid dynamics, and energy homeostasis form an intricate network of metabolic pathways that collectively determine growth performance, tissue development, and overall physiological status in fish. The modulation of these processes through dietary supplements offers promising avenues for improving aquaculture productivity while maintaining fish health and welfare.

Citric acid, a central metabolite in cellular energy production, exerts multifaceted effects on fish metabolism beyond its traditional role in the tricarboxylic acid cycle. Recent investigations have revealed its capacity to influence protein synthesis rates, amino acid catabolism, nucleic acid metabolism, and glucose homeostasis through both direct enzymatic interactions and indirect regulatory mechanisms. Understanding these complex metabolic interactions is crucial for developing evidence-based feeding strategies that maximize growth efficiency while maintaining metabolic homeostasis.

Protein metabolism in fish involves a delicate balance between anabolic and catabolic processes, with tissue-specific variations reflecting functional specialization and metabolic demands. Muscle tissue, comprising the majority of fish body mass, serves as both a primary site of protein synthesis and a reservoir for amino acid mobilization during periods of metabolic stress. Hepatic tissue, functioning as the central metabolic hub, plays crucial roles in protein synthesis, amino acid metabolism, and metabolic regulation. The coordination of protein metabolism between these tissues directly influences growth performance and feed utilization efficiency.

Free amino acid pools in fish tissues represent the immediate precursors for protein synthesis while simultaneously serving as substrates for gluconeogenesis, energy production, and biosynthetic pathways. The dynamic equilibrium between amino acid incorporation into proteins and their catabolic utilization reflects the metabolic state of the organism and provides insights into the efficiency of dietary protein utilization. Alterations in free amino acid profiles following dietary interventions can indicate shifts in metabolic priorities and protein turnover rates.

Nucleic acid metabolism encompasses the synthesis and maintenance of DNA and RNA molecules essential for cellular function, protein synthesis, and genetic information transfer. RNA concentrations, particularly in metabolically active tissues, correlate closely with protein synthesis capacity and cellular activity levels. DNA content, while relatively stable in somatic tissues, may increase during periods of intense growth through cell division and tissue expansion. The coordinated regulation of nucleic acid synthesis reflects cellular metabolic activity and growth potential.

Glucose homeostasis in fish involves complex regulatory mechanisms balancing glucose utilization, glycogen storage, and gluconeogenesis from non-carbohydrate precursors. Blood glucose levels serve as sensitive indicators of metabolic status and dietary intervention effects. The optimization of glucose metabolism through nutritional strategies can enhance energy efficiency and support improved growth performance.

Channa striatus demonstrates unique metabolic characteristics that make it an excellent model for studying nutritional effects on fish metabolism. The species exhibits rapid growth rates, efficient feed conversion, and remarkable metabolic plasticity in response to environmental and nutritional challenges. These attributes, combined with its commercial importance in aquaculture, make it an ideal candidate for investigating the metabolic effects of dietary citric acid supplementation.

Current knowledge gaps include limited understanding of tissue-specific metabolic responses to organic acid supplementation, temporal dynamics of metabolic adaptation, and the integration of protein, amino acid, and nucleic acid metabolism in response to dietary interventions. Furthermore, the dose-response relationships for metabolic parameters remain incompletely characterized across different exposure durations.

This investigation aimed to elucidate the comprehensive metabolic effects of graded citric acid supplementation on protein metabolism, amino acid dynamics, nucleic acid synthesis, and glucose homeostasis in C. striatus. The study tested the hypothesis that citric acid supplementation would enhance protein anabolism, accelerate nucleic acid synthesis, optimize amino acid utilization, and improve glucose metabolism in a dose-dependent manner.

2. Materials and Methods

2.1 Experimental Design and Fish Management

The experimental protocol followed a completely randomized design with four treatment groups: control (0% citric acid), 2% citric acid, 4% citric acid, and 6% citric acid supplementation. Juvenile C. striatus (10 ± 1 g) were randomly allocated to treatment groups in triplicate (n = 30 per treatment) and maintained in 20-liter circular plastic tanks under controlled environmental conditions (27 ± 1°C, natural photoperiod, daily water renewal).

2.2 Diet Preparation and Feeding Protocol

Experimental diets were formulated by incorporating citric acid powder (Sigma Chemical) into commercially available fish meal at concentrations of 2, 4, and 6 g per 100 g basal diet. The fish meal was ground and sieved through 0.5 mm mesh before citric acid integration to ensure homogeneous distribution. Pellets were manufactured using hand pellitization with appropriate moisture content, dried using forced air circulation, and stored in vacuum-sealed containers to prevent degradation.

Fish were fed experimental diets once daily at 2% body weight with weekly feeding rate adjustments based on biomass measurements. Prior to each sampling event, fish were subjected to 24-hour fasting to standardize physiological conditions and minimize digestive content interference with biochemical analyses.

2.3 Sample Collection and Preparation

Tissue samples were collected at 7, 14, 21, and 28 days of experimental feeding. Fish were sacrificed by decapitation following standard protocols, and muscle and liver tissues were rapidly dissected, weighed, and processed for biochemical analysis. Blood samples were collected from the caudal vein using heparinized syringes for glucose determination.

Tissue samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis to preserve biochemical integrity. For each analysis, tissues were homogenized in appropriate buffer solutions using mechanical homogenization with subsequent centrifugation for supernatant collection.

2.4 Protein Analysis

Total protein concentrations in muscle and liver tissues were determined using the Bradford method (Bradford, 1976). Tissue samples (1 g) were homogenized in 5 ml phosphate buffer (pH 7.5) and centrifuged at 8000 rpm for 20 minutes. The supernatant was collected, and appropriate dilutions were prepared for analysis.

The assay was performed by mixing 0.1 ml sample extract with 5 ml Bradford reagent, followed by 2-minute incubation at room temperature. Absorbance was measured at 595 nm using a spectrophotometer against reagent blanks. Protein concentrations were determined using bovine serum albumin standard curves and expressed as mg protein per gram wet tissue weight.

2.5 Free Amino Acid Determination

Free amino acid concentrations were analyzed using the Moore and Stein method (1954) with ninhydrin reagent. Tissue homogenates (5%) were prepared in 10% trichloroacetic acid (TCA) and centrifuged at 1000 rpm for 10 minutes. To 0.5 ml supernatant, 2.0 ml ninhydrin reagent was added, and the mixture was boiled for exactly 6 minutes.

After cooling under running water, the volume was adjusted to 10 ml with distilled water, and absorbance was measured at 570 nm against reagent blanks. Free amino acid concentrations were calculated using appropriate standard curves and expressed as mg amino acids per gram wet tissue weight.

2.6 Nucleic Acid Extraction and Quantification

Nucleic acids were extracted using the Munro and Fleck method (1966) with sequential solvent treatments. Tissue samples were homogenized in absolute methanol, followed by ethanol, ether-ethanol mixture (1:2), 5% TCA, ether, and finally 5% perchloric acid (PCA). The PCA supernatant was used for RNA and DNA quantification.

RNA was determined using the orcinol method with specific color development for pentose sugars. One milliliter PCA extract was mixed with 5 ml orcinol reagent and boiled for 30 minutes. After cooling, absorbance was measured at 560 nm for RNA quantification.

DNA was analyzed using the diphenylamine method specific for 2-deoxypentoses. PCA extract (1 ml) was mixed with 2.5 ml diphenylamine reagent, boiled for 10 minutes, cooled, diluted to 5 ml, and measured at 540 nm for DNA quantification.

2.7 Blood Glucose Analysis

Blood glucose concentrations were determined using the Zarrow method (1964) involving zinc-barium protein precipitation. Blood samples (0.1 ml) were diluted with distilled water, treated sequentially with barium hydroxide and zinc sulfate, centrifuged, and filtered.

The filtrate was processed with copper reagent under standardized boiling conditions, followed by arsenomolybdatecolor development. Glucose concentrations were determined spectrophotometrically at 540 nm using glucose standard curves.

2.8 Statistical Analysis

Data were expressed as mean ± standard error with n = 3 per treatment group. Statistical comparisons between treatments and controls were performed using Student's t-test with significance levels designated as $p < 0.05$ (), $p < 0.01$ (**), and $p < 0.001$ (**). Percentage changes from control values were calculated to facilitate interpretation of treatment effects. Statistical assumptions were verified prior to analysis using appropriate normality and homoscedasticity tests.

3. Results

3.1 Protein Content in Tissues

3.1.1 Muscle Protein Dynamics

Muscle protein concentrations demonstrated remarkable dose-dependent increases across all citric acid treatments, with the most pronounced effects observed at higher concentrations and longer exposure durations. The 6% citric acid treatment exhibited the most dramatic enhancement, with protein levels

increasing progressively from 24.27% at 7 days to 84.86% ($p < 0.001$) at 28 days compared to control values.

The 4% treatment group showed substantial improvements, achieving 10.63% ($p < 0.05$), 20.93% ($p < 0.01$), 33.58% ($p < 0.001$), and 60.75% ($p < 0.001$) increases at 7, 14, 21, and 28 days, respectively. The 2% treatment demonstrated more modest but significant enhancements, particularly at longer durations, with increases of 3.84%, 7.14% ($p < 0.05$), 16.97% ($p < 0.01$), and 22.57% ($p < 0.001$) across the respective time points.

The temporal progression revealed accelerating protein accumulation rates with extended feeding duration, suggesting sustained anabolic activity throughout the experimental period. The dose-response relationship remained consistent across all sampling intervals, with higher citric acid concentrations yielding proportionally greater protein enhancement.

Figure 1

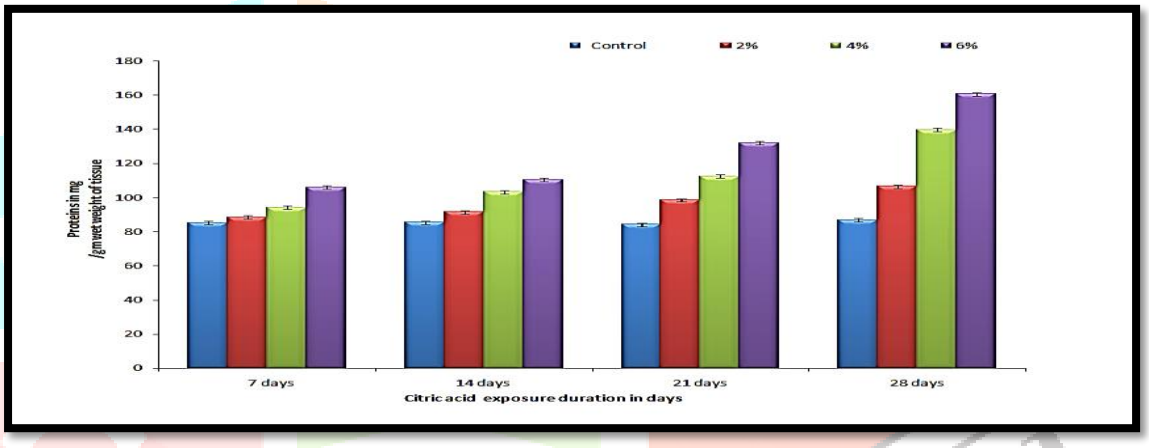


Table 1

% of Citric acid		Days of Exposure			
		7days	14 days	21 days	28 days
Control	Mean	85.26	85.54	84.3	86.95
	SE	3.052	3.257	3.178	3.051
2%	Mean	88.54 NS	91.65*	98.61**	106.58***
	SE	±1.144	±1.04	±1.082	±1.425
	%V	3.84	7.14	16.97	22.57
4%	Mean	94.327*	103.448**	112.608***	139.776***
	SE	±1.077	±1.335	±1.201	±2.903
	%V	10.63	20.93	33.58	60.75
6%	Mean	105.953**	110.432***	132.13***	160.73***
	SE	±2.173	±2.089	±2.768	±2.331
	%V	24.27	29.099	56.74	84.86

Each value is the Mean± SE of six individual Observations.
Values are expressed as mg of protein /gm wet weight of tissue.
SE Standard Error; %V: Percent Variation; NS: Not Significant.
***P<0.05; **P<0.01; ***P<0.001.**

3.1.2 Liver Protein Responses

Hepatic protein concentrations exhibited similar dose-dependent enhancement patterns, though with distinct kinetics compared to muscle tissue. The 6% treatment achieved substantial increases of 36.49% ($p < 0.01$), 35.76% ($p < 0.001$), 46.85% ($p < 0.001$), and 49.14% ($p < 0.001$) at 7, 14, 21, and 28 days, respectively.

The 4% treatment group demonstrated significant improvements across all time points: 16.39% ($p < 0.05$), 23.88% ($p < 0.01$), 41.40% ($p < 0.001$), and 46.43% ($p < 0.001$). The 2% treatment showed more gradual enhancement, with significant increases observed primarily at longer durations: 3.62%, 10.23% ($p < 0.05$), 30.57% ($p < 0.01$), and 34.67% ($p < 0.001$).

The hepatic response pattern indicated rapid initial adaptation to citric acid supplementation, with sustained elevation throughout the experimental period. The liver's central role in metabolic regulation likely contributed to its pronounced responsiveness to dietary citric acid intervention.

Figure 2

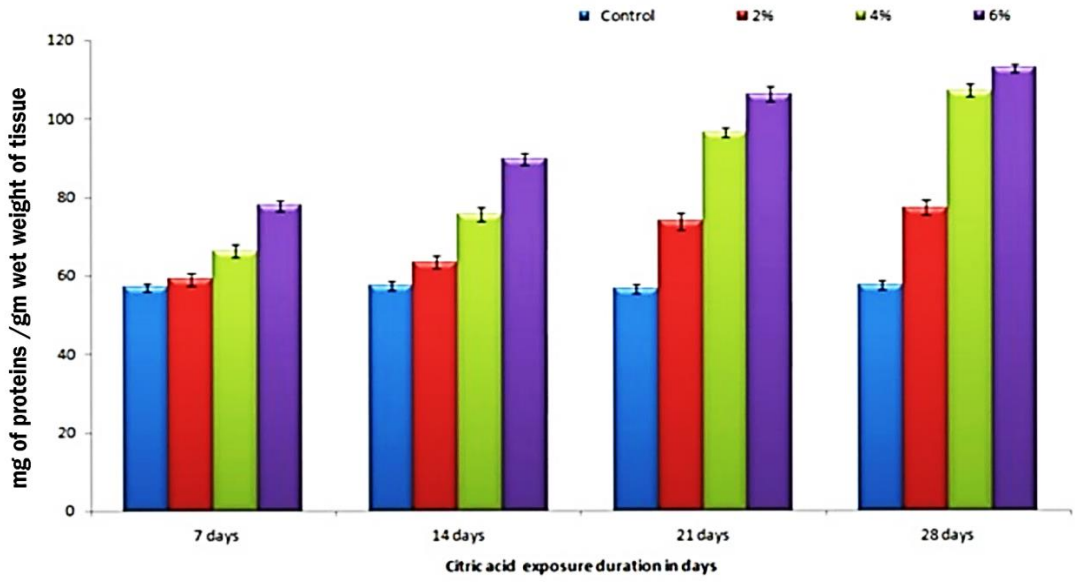


Table 2

% of Citric acid		Days of Exposure			
		7days	14 days	21 days	28 days
Control	Mean SE	57.028 1.070	57.565 1.158	56.461 1.180	57.337 1.182
2%	Mean SE %V	59.090^{NS} ±1.677 3.62	63.454* ±1.494 10.23	73.721** ±2.290 30.57	77.221*** ±1.966 34.68
4%	Mean SE %V	66.38* ±1.745 16.39	75.63** ±1.719 23.88	96.357*** ±1.200 41.40	107.030*** ±1.575 46.43
6%	Mean SE %V	77.84** ±1.238 36.49	89.610*** ±1.418 35.76	106.206*** 1.845 46.85	112.75*** 1.069 49.14

Each value is the Mean± SE of six individual Observations.
Values are expressed as mg of protein/gm wet weight of tissue.
SE Standard Error; %V: Percent Variation; NS: Not Significant.
***P<0.05; **P<0.01; ***P<0.001**

3.2 Free Amino Acid Profiles

3.2.1 Muscle Free Amino Acids

Free amino acid concentrations in muscle tissue decreased progressively with increasing citric acid supplementation, indicating enhanced incorporation into protein synthesis pathways. The 6% treatment demonstrated the most pronounced decreases: -15.53% ($p < 0.05$), -19.78% ($p < 0.05$), -26.49% ($p < 0.01$), and -27.12% ($p < 0.001$) at 7, 14, 21, and 28 days, respectively.

The 4% treatment exhibited significant reductions at most time points: -9.72%, -15.03% ($p < 0.05$), -22.24% ($p < 0.05$), and -22.52% ($p < 0.001$). The 2% treatment showed more modest decreases, with significant reductions primarily at longer durations: -2.74%, -6.31%, -11.05% ($p < 0.05$), and -13.49% ($p < 0.001$).

The inverse relationship between protein accumulation and free amino acid levels provides compelling evidence for enhanced protein synthetic activity in response to citric acid supplementation.

Figure 3

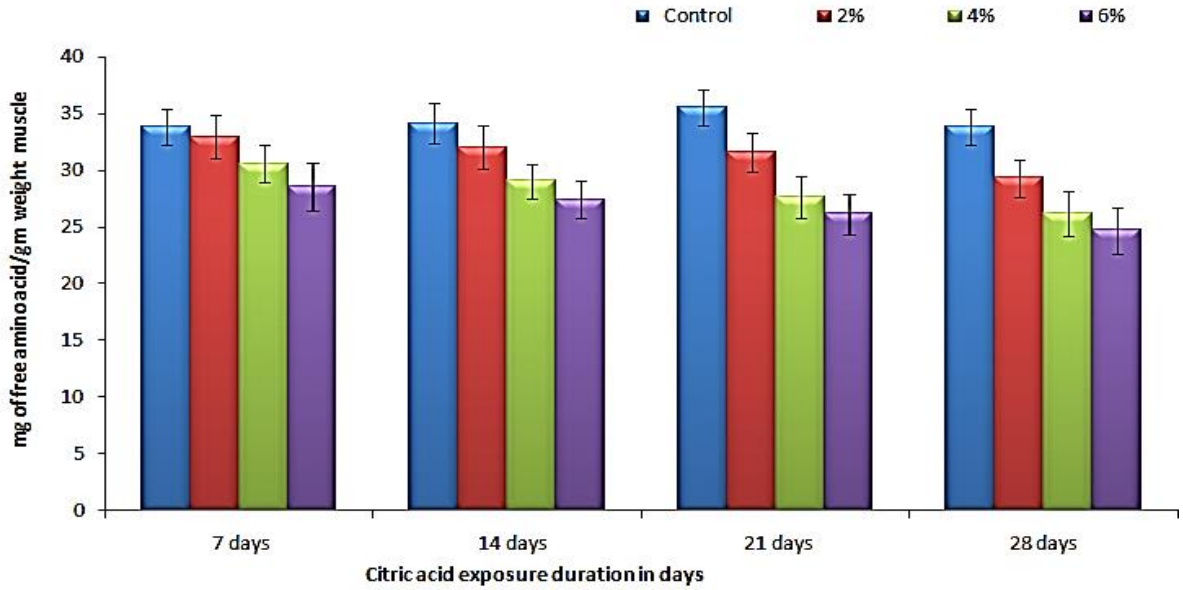


Table 3

% of Citric acid		Days of Exposure			
		7days	14 days	21 days	28days
Control	Mean	33.86	34.22	35.56	33.88
	SE	±0.542	±0.804	±0.554	±0.573
2%	Mean	32.93 NS	32.06 NS	31.63*	29.31*
	SE	±0.573	±0.715	±1.587	±1.256
	%V	-2.74	-6.31	-11.05	-13.49
4%	Mean	30.57 NS	29.075*	27.65*	26.25**
	SE	±1.324	±1.304	±1.53	±1.542
	%V	-9.72	-15.035	-22.24	-22.52
6%	Mean	28.6 *	27.45*	26.14**	24.69***
	SE	±1.233	±1.551	±1.595	±1.541
	%V	-15.53	-19.78	-26.49	-27.12

Each value is the Mean ± SE of six individual Observations
Values are expressed as mg of free amino acid/gm wet weight of tissue.
SE Standard Error; %V: Percent Variation; NS: Not Significant;
***P<0.05; **P<0.01; ***P<0.001.**

3.2.2 Liver Free Amino Acids

Hepatic free amino acid concentrations followed similar declining patterns, though with distinct kinetics reflecting the liver's unique metabolic functions. The 6% treatment achieved substantial reductions: -19.09% ($p < 0.05$), -30.54% ($p < 0.05$), -39.39% ($p < 0.01$), and -44.91% ($p < 0.001$) across the experimental period.

The 4% treatment demonstrated significant decreases at most time points: -10.34%, -24.82% ($p < 0.01$), -28.70% ($p < 0.05$), and -31.52% ($p < 0.01$). The 2% treatment showed progressive reductions with significant effects at longer durations: -6.45%, -16.20%, -17.61% ($p < 0.05$), and -19.03% ($p < 0.05$).

The pronounced decrease in hepatic free amino acids reflects enhanced metabolic flux toward protein synthesis and other anabolic pathways, consistent with the liver's central role in amino acid metabolism.

Figure 4

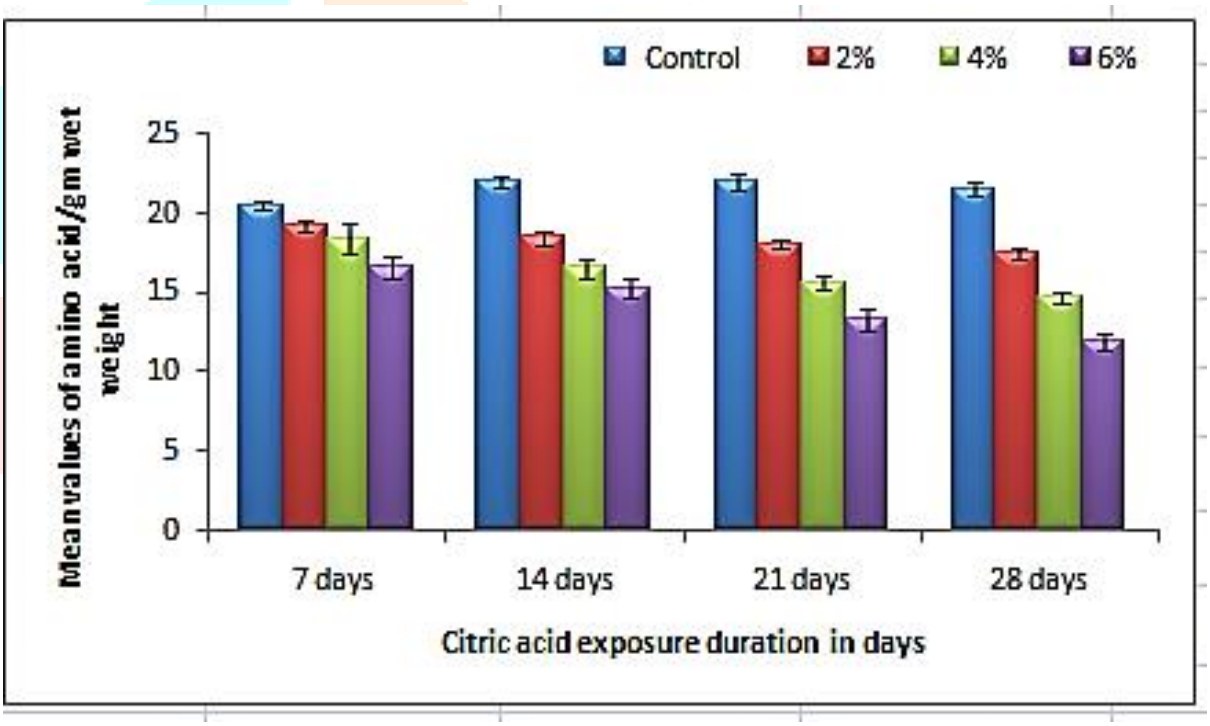


Table 4

% of Citric acid		Days of Exposure			
		7days	14 days	21 days	28 days
Control	Mean	20.44	21.97	21.907	21.51
	SE	±0.268	±0.317	±0.544	±0.473
2%	Mean	19.12 NS	18.41 NS	18.05*	17.415*
	SE	±0.356	±0.388	±0.336	±0.321
	%V	**-6.45**	-16.20	-17.61	-19.038
4%	Mean	18.33 NS	16.51 NS	15.62*	14.73**
	SE	±0.946	±0.57	±0.43	±0.343
	%V	-10.34	-24.82	-28.70	-31.52
6%	Mean	16.54*	15.26*	13.278**	11.85***
	SE	±0.677	±0.63	±0.776	±0.558
	%V	-19.09	-30.54	-39.39	-44.91

3.3 Nucleic Acid Dynamics

3.3.1 DNA Content

DNA concentrations in both muscle and liver tissues showed modest but significant increases, particularly at higher citric acid concentrations and longer exposure durations. In muscle tissue, significant DNA increases were observed primarily with 4% and 6% treatments after 28 days of feeding ($p < 0.05$ for both treatments).

Figure 5

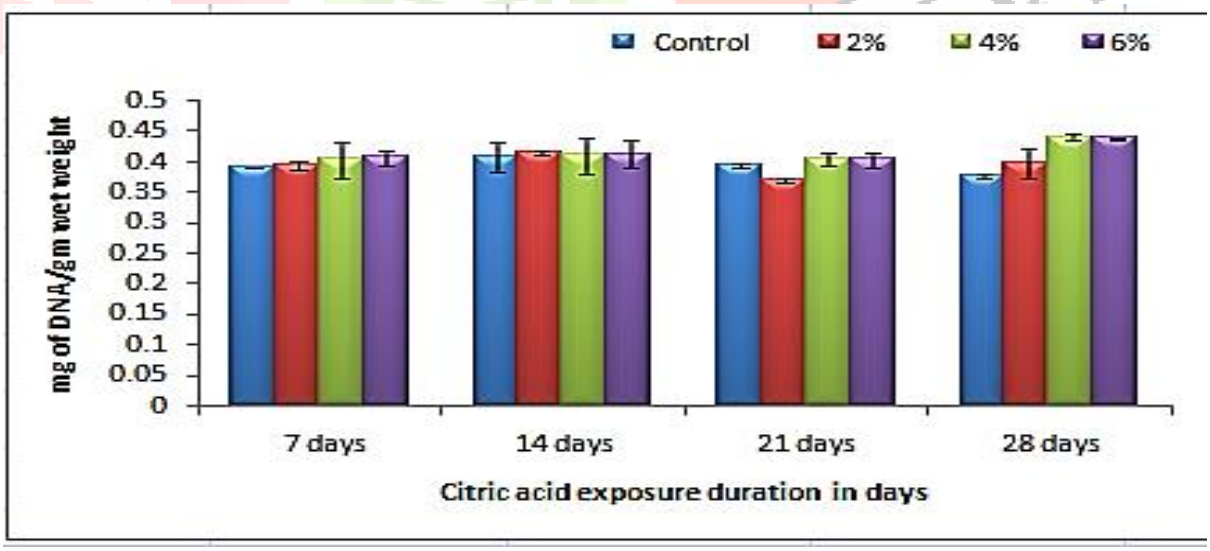


Table 5

% of Citric acid		Days of Exposure			
		7days	14 days	21 days	28 days
Control	Mean SE	0.392 ±0. 21	0.407 ±0.025	0.393 ±0.003	0.377 ±0.0031
2%	Mean SE	0.394 NS ±0.006	0.415 NS ±0.005	0.401 NS ±0.004	0.398 NS ±0.024NS
4%	Mean SE	0.403 NS ±0.03	0.411 NS ±0.03	0.404 NS ±0.010	0.441* ±0.004
6%	Mean SE	0.407 NS ±0.013	0.413 NS ±0.022	0.403 NS ±0.011	0.438* ±0.002

Each value is the Mean± of six individual Observations
Values are expressed as mg of DNA/gm wet weight of tissue.
SE Standard Error; %V: Percent Variation; NS: Not Significant;
***P<0.05; **P<0.01; ***P<0.001**

Hepatic DNA content demonstrated similar patterns, with significant increases observed in the 4% treatment after 28 days ($p < 0.05$) and in the 6% treatment after both 21 and 28 days ($p < 0.05$ for both time points). The modest increases in DNA content suggest enhanced cellular proliferation and tissue growth capacity.

Figure 6

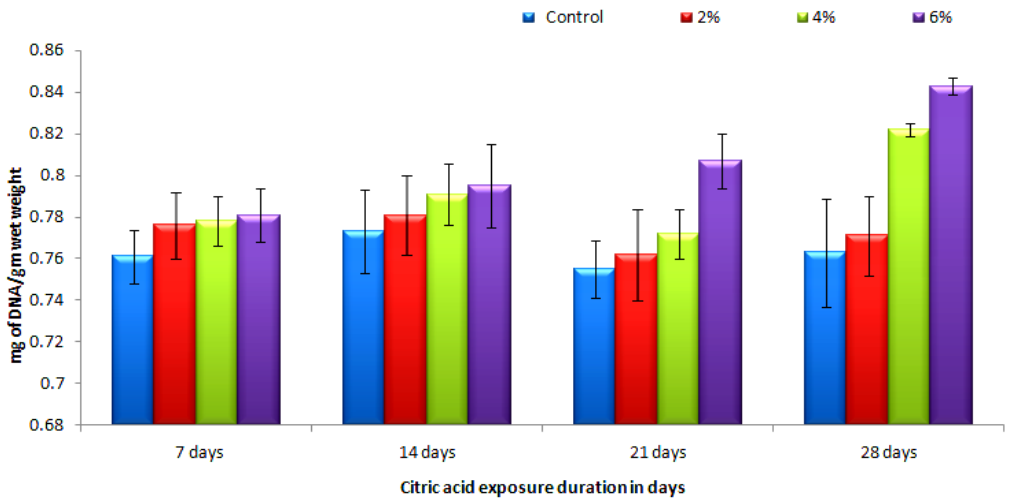


Table 6

% of Citric acid	Days of Exposure				
		7days	14 days	21 days	28 days
control	Mean SE	3.10 ±0.015	3.050 ±0.021	3.150 ±0.015	3.220 ±0.012
2%	Mean SE %V	3.25^{NS} ±0.024 4.62	3.255^{NS} ±0.017 6.300	3.428* ±0.016 8.20	3.61** ±0.006 10.86
4%	Mean SE %V	3.21^{NS} ±0.009 3.42	3.523** ±0.003 13.42	3.66** ±0.052 14.01	3.86*** ±0.071 16.63
6%	Mean SE %V	3.43* ±0.041 9.62	3.71** ±0.067 17.90	3.92*** ±0.016 19.72	4.18*** ±0.045 23.01

3.3.2 RNA Content

RNA concentrations exhibited dramatic dose-dependent increases in both tissues, reflecting enhanced protein synthesis capacity and metabolic activity. In muscle tissue, the 6% treatment achieved remarkable increases of 9.62% ($p < 0.05$), 17.90% ($p < 0.01$), 19.72% ($p < 0.001$), and 23.05% ($p < 0.001$) at 7, 14, 21, and 28 days, respectively.

The 4% treatment demonstrated substantial enhancements: 3.42%, 13.42% ($p < 0.01$), 14.01% ($p < 0.01$), and 16.63% ($p < 0.001$). The 2% treatment showed significant increases at longer durations: insignificant changes at 7 and 14 days, followed by 8.2% ($p < 0.05$) and 10.86% ($p < 0.01$) at 21 and 28 days.

Figure 7

Figure 18 .RNA content in muscle of Channa striatus fed with diet containing different levels of Citric acid

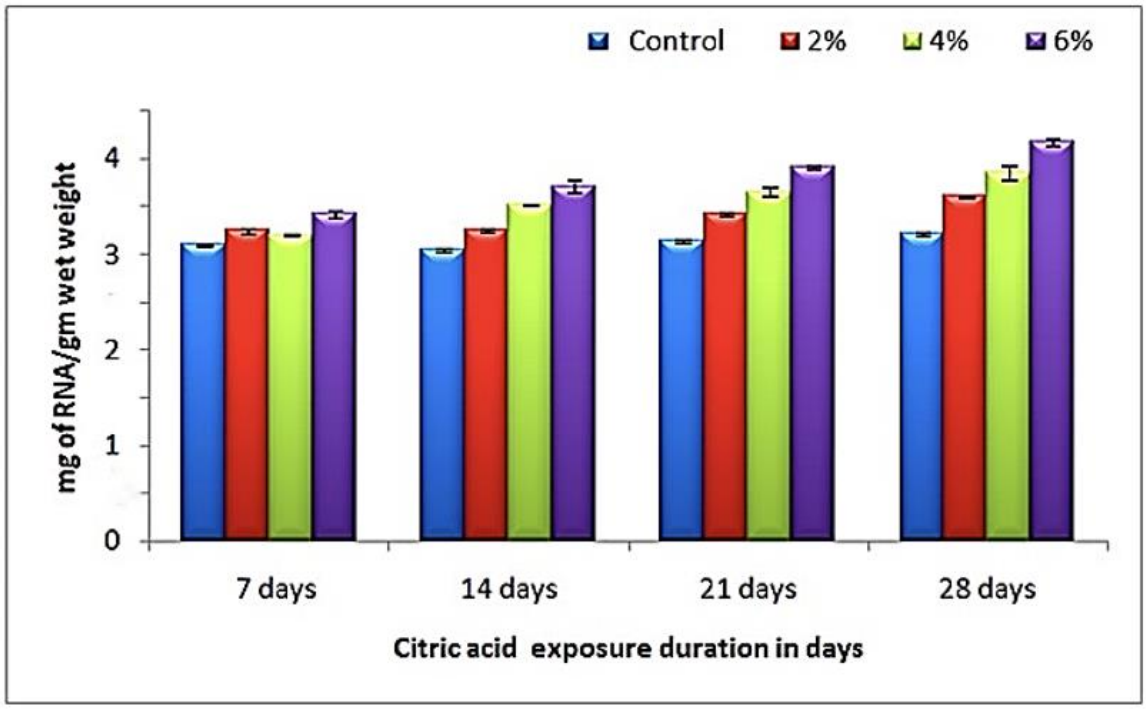


Table 7

% of Citric acid		Days of Exposure			
		7days	14 days	21 days	28 days
Control	Mean	3.890	3.910	3.900	3.930
	SE	±0.052	±0.076	±0.047	±0.022
2%	Mean	4.040^{NS}	4.220*	4.558*	4.83**
	SE	±0.047	±0.017	±0.068	±0.086
	%V	3.71	7.34	14.44	18.63
4%	Mean	4.32^{NS}	4.84**	5.33***	5.9***
	SE	±0.050	±0.045	±0.060	±0.092
	%V	9.95	19.21	26.83	33.39
6%	Mean	4.53**	5.31**	5.737***	6.556***
	SE	±0.034	0.035	±0.029	±0.014
	%V	14.13	26.36	32.02	40.05

Each value is the Mean± of six individual Observations.
Values are expressed as mg of RNA/gm wet weight of tissue.
SE Standard Error; %V : Percent Variation; NS: Not Significant;
*P<0.05; **P<0.01; ***P<0.001.

Hepatic RNA content displayed even more pronounced responses, with the 6% treatment achieving increases of 14.13% ($p < 0.05$), 26.36% ($p < 0.01$), 32.02% ($p < 0.001$), and 40.05% ($p < 0.001$). The 4% treatment showed significant enhancements at most time points: 9.95%, 19.21% ($p < 0.01$), 26.83% ($p < 0.001$), and 33.39% ($p < 0.001$).

Figure 8

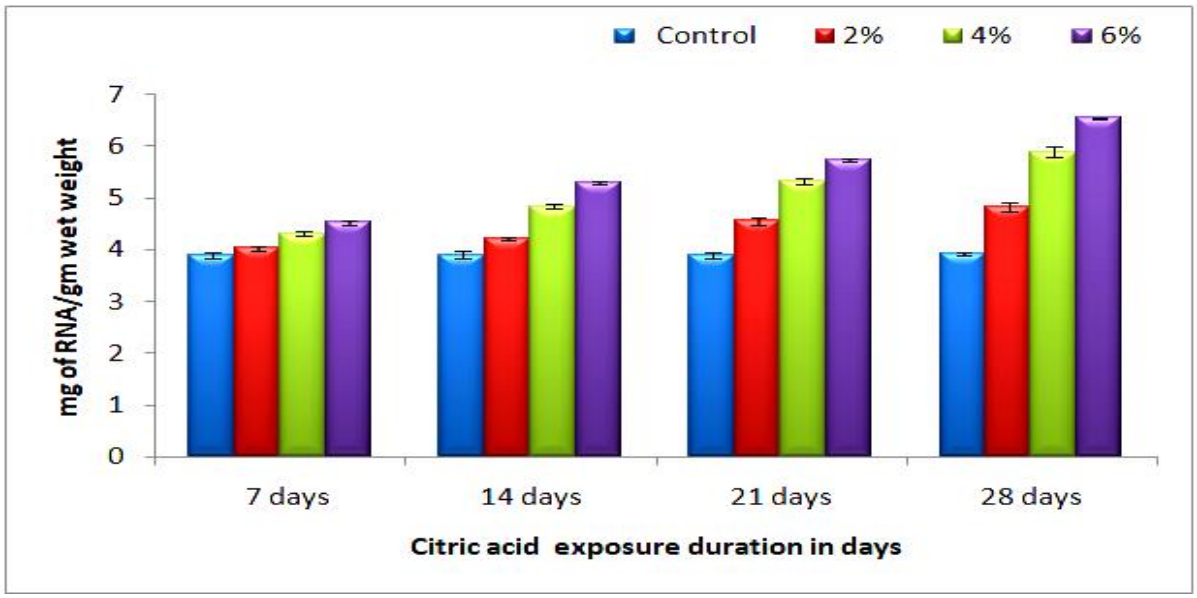


Table 8

% of Citric acid		Days of Exposure			
		7days	14 days	21 days	28 days
Control	Mean SE	54.492 ±1.209	55.322 ±1.186	55.698 ±1.704	54.5 ±1.606
2%	Mean SE %V	51.58NS ±1.586 -3.50	46.45NS ±1.665 -8.80	42.35NS ±1.072 -13.19	39.37* ±1.083 -14.22
4%	Mean SE %V	39.82NS ±1.324 -10.66	37.45NS ±1.294 -14.26	33.83* ±2.065 -17.03	30.78** ±1.155 -19.24
6%	Mean SE %V	28.86* ±1.02 -25.87	26.48* ±1.446 -23.12	24.09** ±1.623 -20.53	22.59*** ±1.542 -22.77

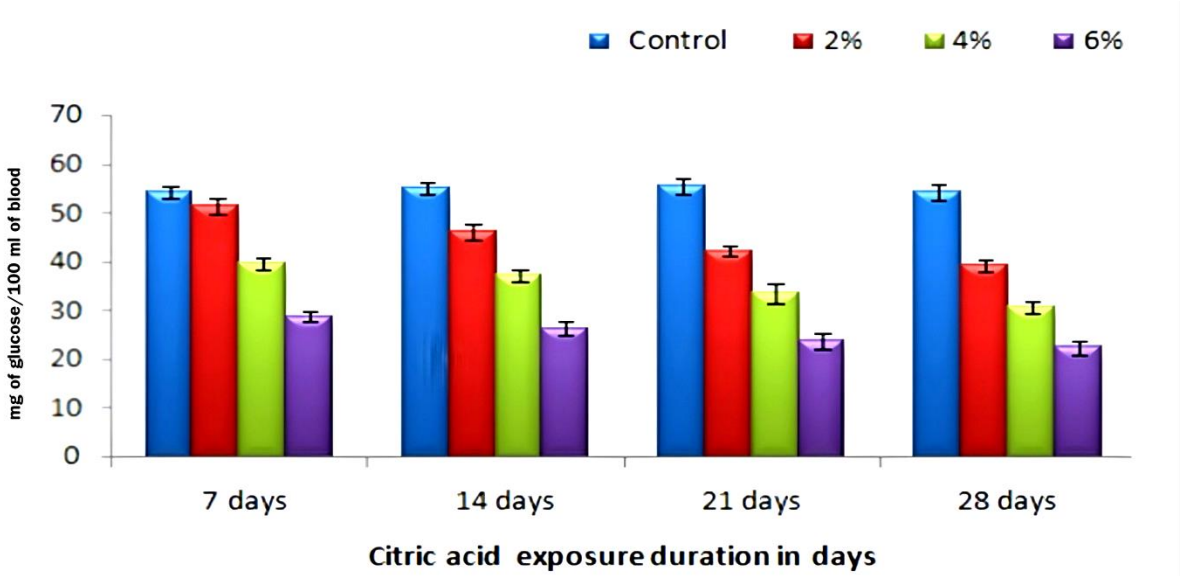
Each value is the Mean ± SE of six individual observations.
Values are expressed as mg of glucose/100 ml of blood. SE - Standard Error, %V- Percent variation, NS: Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001.

The substantial increases in RNA content across both tissues provide strong evidence for enhanced protein synthesis capacity and metabolic activation following citric acid supplementation.

3.4 Blood Glucose Homeostasis

Blood glucose concentrations decreased significantly across all citric acid treatments, indicating improved glucose utilization efficiency and metabolic optimization. The 6% treatment demonstrated the most pronounced reductions: -25.87% ($p < 0.05$), -23.12% ($p < 0.05$), -20.53% ($p < 0.01$), and -22.77% ($p < 0.001$) at 7, 14, 21, and 28 days, respectively.

Figure 9



The 4% treatment achieved substantial decreases: -10.66%, -14.26% ($p < 0.05$), -17.03%, and -19.24% ($p < 0.01$). The 2% treatment showed progressive reductions with significant effects primarily at longer durations: -3.50%, -8.80%, -13.19%, and -14.22% ($p < 0.05$).

The consistent reduction in blood glucose levels across all treatments suggests enhanced glucose uptake and utilization, potentially contributing to improved energy efficiency and growth performance.

4. Discussion

The comprehensive biochemical analysis presented in this study reveals that dietary citric acid supplementation orchestrates a coordinated metabolic reprogramming in C. striatus, characterized by enhanced protein anabolism, accelerated nucleic acid synthesis, and optimized glucose utilization. These metabolic adaptations provide mechanistic insights into the growth enhancement effects observed with citric acid supplementation and demonstrate the compound's multifaceted influence on fish metabolism.

The dramatic increases in tissue protein content, coupled with corresponding decreases in free amino acid pools, provide compelling evidence for enhanced protein synthesis rates following citric acid supplementation. This metabolic shift indicates improved efficiency of amino acid incorporation into structural and functional proteins, contributing directly to growth enhancement and tissue development. The tissue-specific differences observed, with muscle showing the most pronounced protein accumulation, reflect the primary role of skeletal muscle in fish growth and body mass development.

The mechanism underlying citric acid's protein anabolic effects likely involves multiple pathways. As a key intermediate in cellular energy metabolism, citric acid may enhance ATP availability for protein synthesis while simultaneously influencing regulatory pathways controlling translation initiation and elongation. Additionally, citric acid's chelating properties may improve the bioavailability of essential minerals required for protein synthesis enzymes and cofactors.

The remarkable increases in RNA content across both tissues provide strong evidence for enhanced transcriptional activity and protein synthesis capacity. The liver showed particularly pronounced RNA elevation, consistent with its central role in protein synthesis and metabolic regulation. The correlation between RNA increases and protein accumulation supports the interpretation that citric acid supplementation enhances protein synthesis at the translational level.

The modest but significant increases in DNA content, particularly at higher doses and longer durations, suggest that citric acid supplementation may promote cellular proliferation and tissue growth capacity. While DNA increases were less pronounced than RNA changes, they indicate that the growth enhancement effects involve both increased cellular activity and potential hyperplastic growth.

The consistent decreases in blood glucose levels across all treatments demonstrate improved glucose homeostasis and metabolic efficiency. This finding suggests that citric acid supplementation enhances glucose uptake and utilization, potentially through improved insulin sensitivity or enhanced glucose transport mechanisms. The improved glucose metabolism may contribute to enhanced energy availability for anabolic processes and growth.

The dose-response relationships observed across all biochemical parameters support the conclusion that 6% citric acid supplementation represents the optimal concentration within the tested range. The progressive enhancement with increasing doses suggests that higher concentrations may provide additional benefits, though potential adverse effects at excessive doses require consideration.

The temporal progression of metabolic changes reveals that sustained supplementation is required for maximum benefits. The accelerating responses observed with extended feeding duration indicate that metabolic adaptations continue to develop throughout the treatment period, supporting the implementation of long-term supplementation protocols in commercial applications.

The integrated metabolic response observed in this study has significant implications for understanding the mechanisms underlying growth enhancement in aquaculture. The coordinated enhancement of protein synthesis, nucleic acid metabolism, and glucose utilization demonstrates that citric acid supplementation affects multiple levels of cellular metabolism, resulting in comprehensive metabolic optimization.

From a practical aquaculture perspective, these findings support the use of citric acid supplementation as a metabolic enhancer that can improve growth performance through multiple mechanisms. The enhanced protein synthesis capacity, improved amino acid utilization efficiency, and optimized energy metabolism collectively contribute to improved feed conversion and growth rates.

Future research directions should focus on elucidating the molecular mechanisms underlying citric acid's metabolic effects, including investigation of gene expression profiles, enzyme activities, and signalling pathway modulation. Additionally, long-term studies examining the sustainability of metabolic adaptations and potential effects on fish health and product quality would provide valuable insights for commercial implementation.

The economic implications of enhanced metabolic efficiency through citric acid supplementation warrant detailed cost-benefit analysis, considering both the costs of supplementation and the benefits of improved growth performance and feed conversion efficiency. Such analyses would provide crucial information for aquaculture producers considering the adoption of citric acid supplementation protocols.

In conclusion, this study demonstrates that dietary citric acid supplementation induces comprehensive metabolic reprogramming in C. striatus, characterized by enhanced protein anabolism, accelerated nucleic acid synthesis, and improved glucose homeostasis. These metabolic adaptations provide mechanistic explanations for the growth enhancement effects of citric acid supplementation and support its application as a metabolic optimizer in sustainable aquaculture systems.

5. Conclusion

This investigation provides unprecedented insights into the metabolic mechanisms underlying citric acid-induced growth enhancement in Channa striatus, revealing a coordinated reprogramming of cellular metabolism that optimizes anabolic processes and energy utilization. The comprehensive biochemical analysis demonstrates that citric acid supplementation orchestrates fundamental changes in protein metabolism, nucleic acid dynamics, and glucose homeostasis that collectively contribute to enhanced growth performance and physiological efficiency.

The dramatic 84.86% increase in muscle protein content and 49.14% enhancement in liver protein concentrations, coupled with corresponding decreases in free amino acid pools, provide compelling evidence for enhanced protein synthetic capacity. This metabolic shift represents a fundamental optimization of nitrogen utilization, converting dietary amino acids more efficiently into structural and functional proteins essential for growth and development.

The remarkable increases in RNA content, reaching 40.05% in liver tissue with 6% citric acid supplementation, demonstrate enhanced transcriptional activity and protein synthesis machinery development. This molecular-level enhancement provides the cellular infrastructure necessary to support increased protein synthesis rates and metabolic activity, establishing the biochemical foundation for improved growth performance.

The consistent decreases in blood glucose levels across all treatments, achieving reductions of up to 22.77% with optimal supplementation, indicate improved glucose utilization efficiency and metabolic

optimization. This enhancement in glucose homeostasis suggests better energy allocation toward anabolic processes, contributing to overall metabolic efficiency and growth enhancement.

The dose-response relationships observed across all biochemical parameters provide clear evidence that 6% citric acid supplementation represents the optimal concentration for metabolic enhancement within the tested range. The progressive improvements with increasing concentrations, combined with the absence of adverse metabolic effects, support the safety and efficacy of this supplementation level for commercial applications.

The temporal dynamics revealed in this study demonstrate that metabolic adaptations continue to develop throughout extended supplementation periods, with maximum benefits achieved after 28 days of feeding. This finding has important implications for commercial feeding protocols, suggesting that sustained supplementation strategies are necessary to realize the full metabolic benefits of citric acid intervention.

From a mechanistic perspective, the integrated metabolic response observed in this study suggests that citric acid functions as a metabolic modulator affecting multiple levels of cellular organization. The coordinated enhancement of protein synthesis, nucleic acid metabolism, and glucose utilization indicates that citric acid influences fundamental regulatory pathways controlling cellular anabolism and energy metabolism.

The practical implications of these findings extend beyond basic scientific understanding to provide actionable insights for aquaculture nutrition. The enhanced metabolic efficiency achieved through citric acid supplementation translates directly into improved feed conversion ratios, reduced production costs, and enhanced profitability for commercial operations.

The environmental benefits of metabolic optimization through citric acid supplementation align with sustainable aquaculture goals. Improved nutrient utilization efficiency reduces waste production and environmental impact while maintaining or enhancing production output, contributing to more sustainable intensification of aquaculture systems.

These findings establish citric acid supplementation as a scientifically validated metabolic enhancement strategy with broad applications in aquaculture nutrition. The fundamental nature of the metabolic processes affected suggests that similar benefits may be achievable across diverse fish species, expanding the potential impact of this nutritional intervention.

The research presented here advances our understanding of fish metabolism and nutritional physiology while providing practical solutions for aquaculture optimization. The comprehensive approach, examining multiple metabolic systems across extended time periods, establishes a robust foundation for future research and commercial application.

Future investigations building on these findings should explore the molecular mechanisms underlying citric acid's metabolic effects, examine species-specific responses, and evaluate long-term sustainability of metabolic adaptations. Such studies will further refine our understanding and optimize practical applications of citric acid supplementation in aquaculture.

In conclusion, this study establishes dietary citric acid supplementation as a powerful metabolic optimizer that enhances protein anabolism, accelerates nucleic acid synthesis, and improves glucose homeostasis in C. striatus. The comprehensive metabolic reprogramming achieved through optimal 6% supplementation provides mechanistic insights into growth enhancement while offering practical solutions for sustainable aquaculture intensification. These findings represent a significant advancement in aquaculture nutrition science and provide a foundation for developing next-generation feeding strategies that optimize fish metabolism for enhanced productivity and sustainability.

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