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Effect of *N*-acetyl cysteine and glycine supplementation on growth performance, glutathione synthesis, anti-oxidative and immune ability of Nile tilapia, *Oreochromis niloticus*

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ABSTRACT

An 8-week feeding trial was conducted to evaluate the effect of *N*-acetyl cysteine (NAC) and glycine supplementation on growth performance, glutathione (GSH) synthesis, anti-oxidative and immune ability of Nile tilapia, *Oreochromis niloticus*. Four practical diets were formulated, control, control +0.2% NAC, control +0.5% glycine, control +0.2% NAC +0.5% glycine. Each diet was randomly assigned to quadruplicate groups of 30 fish (approximately 9.5 g). The weight gain and specific growth rate were significantly increased with the supplementation of NAC and glycine. While they had no effect on feed efficiency feed intake and survival. Glutathion peroxidase (GPx) was increased by NAC and γ-glutamine cysteine synthase (γ-GCS) in plasma were increased by glycine. After the feeding trail, fish were challenged by *Streptococcus iniae*, fish fed the diet supplemented with NAC obtained significantly higher survival rate after 72 h challenge test. NAC also decreased malonaldehyde (MDA) in liver, increased glutathione S-transferase (GST) activity in plasma, up-regulated mRNA expression of Superoxide dismutase (SOD) and GPx in liver and headkidney. Dietary supplementation of glycine increased the anti-oxidative ability of tilapia through increase anti-oxidative enzyme activity (SOD, glutathione reductase, myeloperoxidase) and up-regulate anti-oxidative gene expression (SOD). Immune ability only enhanced by the supplementation of NAC through increased interleukin-1β (IL-1β) mRNA expression. These results clearly indicated that the supplementation of NAC and glycine can significantly improve the growth performance of tilapia, and NAC also enhance the anti-oxidative and immune capacity of tilapia, glycine could only enhance the anti-oxidative ability.

1. Introduction

Tilapia is one of the most important and extensively cultured fish species around the globe. Among all the tilapia species, Nile tilapia (*Oreochromis niloticus*) is the most important one because of its rapid growth, high yield, delicious taste and low price [1]. But nowadays, the rapid development of aquaculture resulted in many problems such as diseases and the deterioration of environment, especially in China. *Streptococcus iniae* have been regarded as one of the most important pathogen in tilapia, bring out massive mortality and economic losses in aquaculture [2,3].

Traditionally, Amino acids (AA) were classified as nutritionally essential or nonessential based on nitrogen balance or growth. AA whose carbon skeletons are not synthesized *de nove* by animals or humans must be provided in diets, therefore, considered nutritionally essential. In contrast, AA that can be synthesized *de novo* in animals are thought to be nutritionally nonessential [4,5]. But it was tactically assumed and without much evidences that animals or humans could synthesize sufficient amounts of all non-essential AA (NEAA) and did not need them in diets for optimal nutrition or health [6,7]. Recently, there has been growing evidences from cell culture and animal studies shows that some of the traditionally classified NEAA (e.g. glycine, glutamine, cysteine, taurine and proline) are important regulators of key metabolic pathways, which play enormous roles in multiple signaling pathways, thereby regulating gene expression, intracellular protein turnover, nutrient metabolism, and oxidative defense. These have led to the development of the concept of functional AA (FAA). In this study, we will test two of them, cysteine and glycine.

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The redox state of the microenvironment represents a fine-tuned balance between oxidant and anti-oxidative activity, whereby the tripeptide Glutathione (GSH), consisting of glutamate, cysteine, and glycine, constitutes a large part of the factors determining the intracellular redox balance [8], among the anti-oxidative defense systems, GSH is the most ubiquitous and abundantly available within animal cells, a lower concentration of GSH is indicative of impaired antioxidant capacity [9]. Besides that, GSH also plays an important role in detoxification of xenobiotics, cell proliferation and immune functions [10,11]. The recent research about GSH synthesis is mainly on cysteine and glycine, not glutamate, because the physiological concentration of glutamate is extremely high, and catabolic processes of virtually all amino acids cycle through glutamate production [12]. Cysteine and glutamate are first to form a dipeptide r-glutamyl-cysteine, catalyzed by r-glutamylcysteine synthetase (γ-GCS), then GSH synthetase catalyze the dipeptide reacts with glycine to produce GSH. For many years, cysteine and γ-GCS have been considered to be the rate-limiting precursor and enzyme of in vivo GSH synthesis [13–15], and since cysteine is rapidly oxidized and is also toxic at high concentrations, it is not practical to use cysteine as a supplement, *N*-acetyl cysteine (NAC) has been the most widely used cysteine precursor due to its fast utilization [8,16]. Although overexpression of GSH synthetase or excess glycine failed to increase GSH levels [14], however, deficiency of them would result in GSH deficiency, especially under stress conditions [13,17]. Growing evidence shows that glycine also plays a crucial role in metabolic regulation, anti-oxidative reactions and neurological function [18]. Glycine is insufficient in plant protein based diet, and our latest research has shown that glycine is not sufficient for *Litopenaeus vannamei* fed with low fish meal diet [19], so we can assume that glycine is also insufficient to meet tilapia's requirement based on its diet composition.

The aim of this study is to evaluate the effect of dietary NAC and glycine on growth performance, anti-oxidative reaction, immune response and bacterial challenge of juvenile Nile tilapia.

2. Materials and methods

2.1. Diet preparation

Composition of the mainly ingredients were presented in Table 1. Four practical diets (control, NAC, glycine, NAC + glycine) were formulated (Table 2). The diets were supplemented with lysine and methionine to satisfy the requirement for juvenile tilapia [1,20]. All the dry ingredients were finely ground, weighed, and then well-mixed by a Hobart mixer (A-200T Mixer bench Model unit, Russell Food Equipment, Ottawa, ON, Canada). Lipids and water were then added and thoroughly mixed. The 1.5 mm and 2.5 mm diameter pellets were cold-extruded using a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangdong, China), then air-dried to approximately 10% moisture, and stored at −20 °C until used.

Table 1
Composition of the mainly ingredients (%).

	Soybean meal	Rapeseed meal	Wheat flour	Peanut meal
Crude protein	48.8	36.33	12.12	45.81
Lys	2.83	1.955	0.25	1.53
Thr	1.89	1.57	0.31	1.2
Met	0.65	0.74	0.2	0.53
Cys	0.65	0.77	0.27	0.6
Trp	0.37	0.5	0.12	0.45
Ile	2.19	1.45	0.42	1.53
Val	2.26	1.84	0.49	1.88
Leu	3.57	2.48	0.79	2.84
Phe	2.39	1.44	0.55	2.25
Tyr	1.5	1	0.13	1.8
His	1.19	0.99	0.26	1.05
Arg	3.42	2.1	0.45	4.91
Ala	2.11	1.65	0.36	1.79
Asp	5.51	2.6	0.49	5.2
Glu	8.4	6.07	3.85	8.51
Gly	2.04	1.81	0.41	2.6
Ser	2.44	1.53	0.53	2.14
Pro	2.25	2.24	1.31	1.92
TAA[a]	45.66	32.735	11.19	42.73

[a] TAA: Total amino acids.

2.2. Animals and experimental conditions

Juvenile tilapias were obtained from a commercial hatchery (Guangzhou, China). Prior to start the experiment, the fish were acclimated to the culture environment for 3 weeks and fed with a commercial diet (obtained from Zhongshan Taishan Feed Co., Ltd. Guangdong, China). At the beginning of the experiment, 480 fish of the similar size were distributed randomly into 16 fiberglass tanks (200 L, 30 fish for each tank, 4 tanks per diet), the initial body weight was approximately 9.5 g. All groups of fish were fed three times daily at 9:00, 13:00 and 17:00 with 6–8% body weight per day for 8 weeks. During the feeding trial, fish were weighed every 2 weeks and the amount of diet given was adjusted accordingly. Any uneaten feeds were collected by siphoning, then used to calculate feed intake.

During the experimental period, they were provided with a continuous flowof water (200 ml s^{-1}), water temperatures ranged from 27 to 30 °C, pH 7.0–7.2, the ammonia nitrogen was lower than

Table 2
Formulation and proximate composition of experimental diets (% dry matter).

Ingredients	Control	NAC	Glycine	NAC + glycine
Soyben meal[a]	18	18	18	18
Fish meal[a]	2	2	2	2
Rapeseed meal[a]	32	32	32	32
Wheat flour[a]	23.5	23.3	23	22.8
Rice bran meal[a]	5	5	5	5
Peanut meal[a]	10	10	10	10
a-starch[a]	3	3	3	3
Monocalcium phosphate[b]	2	2	2	2
Soy oil[a]	1	1	1	1
Soy lecithin[a]	1	1	1	1
Vitamin mixture[c]	1	1	1	1
Mineral mixture[d]	1	1	1	1
Choline chloride (50%)[b]	0.2	0.2	0.2	0.2
Ascorbic acid Polyphosphate[b]	0.1	0.1	0.1	0.1
L-lysine hydrochloride (78%)[e]	0.1	0.1	0.1	0.1
DL-Met[f]	0.1	0.1	0.1	0.1
NAC[e]	0	0.2	0	0.2
Glycine[e]	0	0	0.5	0.5
Proximate composition (% dry matter)				
Dry matter	89.41	89.48	90.29	89.88
Crude protein	33.89	34.04	34.50	34.72
Crude lipid	4.10	4.08	4.03	3.96
Ash	8.34	8.31	8.48	8.37
Glycine	1.49	1.48	1.94	1.93
Cysteine	0.56	0.73	0.56	0.75

[a] Supplied by Haida Feed Corporation, Guangzhou, China.
[b] Supplied by Ashare, Guangzhou, China.
[c] Vitamin mixture (IU or mg g^{-1} of diet): vitamin A, 6000 IU; vitamin D3, 5600 IU; vitamin E, 0.04; vitamin K3, 10; vitamin B1, 9; vitamin B2, 18; vitamin B6, 12; vitamin B12, 0.04; vitamin C, 140; niacin, 70; biotin, 0.16; folic acid, 3.2.
[d] Mineral mixture (mg g^{-1} of diet): D-calcium pantothenate, 40; magnesium, 100; iron, 70; manganese, 13.3; iodine, 2.24; copper, 10.5; zinc, 56; selenium, 0.3; cobalt, 1.75.
[e] Supplied by Aladdin Industrial Corporation, Shanghai, China.
[f] Supplied by Evonik, Beijing, China.