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Deficiency of dietary niacin impaired gill immunity and antioxidant capacity, and changes its tight junction proteins via regulating NF-κB, TOR, Nrf2 and MLCK signaling pathways in young grass carp (*Ctenopharyngodon idella*)

Shun-Quan Li [a, 1], Lin Feng [a, b, c, 1], Wei-Dan Jiang [a, b, c], Yang Liu [a, b, c], Jun Jiang [a, b, c], Pei Wu [a, b, c], Sheng-Yao Kuang [d], Ling Tang [d], Wu-Neng Tang [d], Yong-An Zhang [e], Xiao-Qiu Zhou [a, b, c, *]

[a] *Animal Nutrition Institute, Sichuan Agricultural University, Chengdu, 611130, China*
[b] *Fish Nutrition and Safety Production University Key Laboratory of Sichuan Province, Sichuan Agricultural University, Chengdu, 611130, China*
[c] *Key Laboratory for Animal Disease-Resistance Nutrition of China Ministry of Education, Sichuan Agricultural University, Chengdu, 611130, China*
[d] *Animal Nutrition Institute, Sichuan Academy of Animal Science, Chengdu, 610066, China*
[e] *Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China*

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ABSTRACT

To investigate the effects of dietary niacin on gill immunity, tight junction proteins, antioxidant system and related signaling molecules mRNA expression, young grass carp (*Ctenopharyngodon idella*) were fed six diets containing graded levels of niacin (3.95–55.01 mg/kg diet) for 8 weeks. The study indicated that niacin deficiency decreased lysozyme and acid phosphatase activities, and complement 3 content, and caused oxidative damage that might be partly due to the decreased copper, zinc superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and glutathione-S-transferase activities and reduced glutathione content in fish gills ($P < 0.05$). Moreover, the relative mRNA levels of antimicrobial peptides (liver expressed antimicrobial peptide 2 and Hepcidin), anti-inflammatory cytokines (interleukin 10 and transforming growth factor β1), tight junction proteins (Occludin, zonula occludens 1, Claudin-15 and -3), signaling molecules (inhibitor of κBα (IκBα), target of rapamycin (TOR), ribosomal protein S6 kinase 1 (S6K1) and NF-E2-related factor 2 (Nrf2)) and antioxidant enzymes were significantly decreased ($P < 0.05$) in niacin-deficient diet group. Conversely, the mRNA levels of pro-inflammatory cytokines (tumor necrosis factor α, interleukin 8, interferon γ2, and interleukin 1β), signaling molecules (nuclear factor kappa B p65, IκB kinase α, IκB kinase β, IκB kinase γ, Kelch-like-ECH-associated protein 1b, myosin light chain kinase and p38 mitogen-activated protein kinase (p38 MAPK) were significantly increased ($P < 0.05$) in fish gills fed niacin-deficient diet. Interestingly, the varying niacin levels of 3.95–55.01 mg/kg diet had no effect on the mRNA level of Kelch-like-ECH-associated protein 1a, Claudin-c and −12 in fish gills ($P > 0.05$). In conclusion, niacin deficiency decreased gill immunity, impaired gill antioxidant system, as well as regulated mRNA expression of gill tight junction proteins and related signaling molecules of fish.

1. Introduction

In fish, the gill is one of the largest immune organs [1] and plays a central role in the physiology of fish (such as gas exchange, acid-base balance, osmoregulation and nitrogenous waste excretion) [2]. Moreover, these physiology function of fish gill results in water-borne antigens (such as bacteria, fungi, viruses and other toxic elements) exposing to gill and thus leading to immune response [3].

* Corresponding author. Animal Nutrition Institute, Sichuan Agricultural University, Chengdu, 611130, China.
E-mail addresses: xqzhouqq@tom.com, zhouxq@sicau.edu.cn (X.-Q. Zhou).
[1] These two authors contributed to this work equally.

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It was revealed that fish have gill immune and physical barrier function, which fighted against these potentially dangerous antigens in the gill [3]. Chen et al. [4] showed that disturbance of the gill immune and physical barrier function could cause fish impaired immune response and growth retardation. Moreover, gill immune and physical barrier function were related to nutrients in fish [4,5]. To our knowledge, niacin is an indispensable water-soluble vitamin for fish [6]. However, to date, no report has addressed the effects of dietary niacin deficiency on gill immune and physical barrier function in fish. Study in *Corynebacterium crenatum* showed that niacin deficiency decreased the synthesis of arginine [7]. Arginine deficiency impaired gill immune and physical barrier function [3]. Moreover, our previous study showed that dietary niacin deficiency decreased grass carp growth [8]. Fish growth is positively correlated with the gill immune and physical barrier function [3]. These data indicated a possible correlation between niacin and gill immune and physical barrier function in fish, which is valuable for investigation.

In fish, the gill immune function partly relied on their immune response [9] associated with lysozyme (LA), acid phosphatase (ACP), complement (C3) and antimicrobial peptides [10]. However, information regarding the effects of niacin on immune response in fish gill is scarce. Study in rat showed that niacin deficiency could increase the cortisol content [11]. It was revealed that cortisol could inhibited proliferation of monocyte/macrophage cell line in rainbow trout [12] and decreased macrophages survival of Atlantic salmon (*Salmo salar Linnaeus*) when exposed to the bacterium [13]. Additionally, monocytes and macrophages could synthesize the antibacterial compounds like LA, ACP and C3 in mammals [14]. Meanwhile, study from our laboratory showed that niacin-limited diet could decrease LA, ACP activities and C3 content in serum of juvenile Jian carp [15]. These observations indicate that niacin may have beneficial effects on gill immune response of fish, which is worth of further systematic studying. In fish, the gill immune response is also primarily mediated by cytokines namely tumor necrosis factor α (TNF-α), interleukin 8 (IL-8), interleukin 1β (IL-1β), interleukin 10 (IL-10) and transforming growth factor-β (TGF-β) [4]. Moreover, production of cytokine could be involved in signaling pathway of nuclear factor κB (NF-κB) [16] and TOR [17]. Study in grass carp (*Ctenopharyngodon idella*) showed that NF-κB inhibition reduced the release of TNF-α in the head kidney leukocytes [18]. Besides, it was revealed that inhibition of TOR could cause the increase of TNF-α production and reduced the release of IL-10 in human [17]. However, no reports at present have addressed the role of NF-κB and TOR in regulating production of cytokines in fish by niacin. Study in yeasts showed that niacin deficiency decreased the glutamine synthesis [19]. Glutamine deficiency could increase NF-κB P65 expression [20] in Caco-2 cells and decrease TOR expression for fish [21]. These data indicated niacin might be affecting the regulation of cytokine by modulating NF-κB and TOR signaling, which need to be investigated.

In fish, the gill immune function also in part depended on its barrier function, which is related to gill structure integrity and the tight junction (TJ) complex between its epithelial cells [4,22]. Study in freshwater fish showed that gill was prone to oxidative damage, which could disturb structural integrity of epithelial cells [23]. To protect the gill against the oxidative damage, fish have developed antioxidant system which includes non-enzymatic antioxidant compounds and enzymatic antioxidant compounds [4]. It is reported that the enzyme activity change was partly depended on enzyme gene expression in rat tissue [24]. The gene expression of antioxidant enzymes proved to be regulated by NF-E2-related factor 2 (Nrf2) and its cytosolic repressor Kelch-like-ECH-associated protein 1 (Keap1) in fish [3]. Recently, our laboratory was the first to clone the cDNA of Nrf2 (GenBank accession no. KF733814), Keap1a (GenBank accession no. KF811013) and Keap1b (GenBank accession no. KJ729125) of grass carp (*Ctenopharyngodon idella*). To date, there is no information at present regarding the role of Nrf2 signaling pathway in regulating gene expression of antioxidant enzymes in animal by niacin. In fish gill, TJ complex consists of both transmembrane and cytosolic proteins, such as Occludin, Claudins and zonula occludens 1 (ZO-1) [25]. However, no study has focused on the effects of niacin on TJ proteins in animal. In yeasts, it was reported that niacin increased the glutamine synthesis [19]. Study in Caco-2 cell monolayer showed glutamine could prevented the disruption of ZO-1 and occludin thus protecting barrier function [26]. Thus, these data indicate a possible correlation between niacin and TJ proteins in animals, which remains to be elucidated.

This study used the same growth trial as the previous study [8]. From the foregoing statement, the present study was the first report to investigate the effects of niacin on gill immune barrier, gill physical barrier and related signal molecules involved in NF-κB, TOR and Nrf2 signaling pathways in fish, which could provide partial theoretical evidence for the effects of niacin on fish gill health status.

2. Materials and methods

2.1. Experimental design and diets

The basal diet formulation was the same as our previous study and presented in Table 1 [8]. Fish meal, gelatin and casein were used as the main protein sources. The dietary protein level was fixed at 30%, as described by Khan et al. [27]. The mixture of vitamin without niacin was prepared as the method described by Jiang et al. [28]. The niacin concentrations of the six diets were 3.95 (unsupplemented), 14.92, 24.98, 35.03, 44.97 and 55.01 mg/kg diet determined by high-performance liquid chromatography (HPLC)

Table 1
Composition and nutrient content of the basal diet.

Ingredients	g kg^{-1} diet	Nutrients content	g kg^{-1} diet
Fishmeal	37.50	Crude protein[d]	293.20
Casein	248.10	Crude lipid[d]	45.40
Gelatin	75.00	n-3[e]	10.00
DL-Met (99%)	1.40	n-6[e]	10.00
Alpha-starch	240.00	Available phosphorus[e]	6.00
Corn starch	226.20		
Fish oil	25.00		
Soybean oil	18.90		
Vitamin premix[a]	10.00		
Mineral premix[b]	20.00		
Niacin premix[c]	15.00		
$Ca(H_2PO_4)_2$	22.40		
Choline chloride (60%)	10.00		
Cellulose	50.00		
Ethoxyquin (30%)	0.50		

[a] Vitamin premix (g kg^{-1} premix): retinyl acetate (500,000 IU g^{-1}), 2.40; cholecalciferol (500,000 IU g^{-1}), 0.40; D,L-a-tocopherol acetate (50%), 12.54; menadione (23%), 0.79; thiamine nitrate (98%), 0.04; calcium-D-pantothenate (98%), 2.43; pyridoxine hydrochloride (98%), 0.59; cyanocobalamin (1%), 0.81; riboflavin (80%), 0.55; D-biotin (2%), 4.91; mesoinositol (99%), 19.19; folic acid (96%), 0.40; ascorbyl acetate (93%), 7.16. All ingredients were diluted with corn starch to 1 kg.

[b] Mineral premix (g kg^{-1} premix): $MgSO_4 \cdot H_2O$, 56.200; $FeSO_4 \cdot H_2O$, 22.900; $CuSO_4 \cdot 5H_2O$, 0.020; $ZnSO_4 \cdot H_2O$, 0.630; $MnSO_4 \cdot H_2O$, 1.650; KI, 0.070; $NaSeO_3$, 0.004. All ingredients were diluted with corn starch to 1 kg.

[c] Niacin premix (mg kg^{-1} premix): premix was added to obtain graded levels of niacin. The final niacin concentrations in each experimental diet were determined to be 3.95, 14.92, 24.98, 35.03, 44.97 and 55.01 mg kg^{-1} diet, respectively.

[d] Crude protein, crude lipid and total phosphorus contents were measured value.

[e] Available phosphorus, n-3 and n-6 contents were calculated according to NRC (2011).