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Dietary pantothenic acid deficiency and excess depress the growth, intestinal mucosal immune and physical functions by regulating NF-κB, TOR, Nrf2 and MLCK signaling pathways in grass carp (*Ctenopharyngodon idella*)

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ABSTRACT

This study investigated the effects of dietary pantothenic acid (PA) on the growth, intestinal mucosal immune and physical barrier, and relative mRNA levels of signaling molecules in the intestine of grass carp (*Ctenopharyngodon idella*). A total of 540 grass carp (253.44 ± 0.69 g) were fed six diets with graded levels of PA (PA1, PA15, PA30, PA45, PA60 and PA75 diets) for 8 weeks. The results indicated that compared with PA deficiency (PA1 diet) and excess (PA75 diet) groups, optimal PA supplementation increased ($P < 0.05$): (1) percent weight gain (PWG), feed intake and feed efficiency; (2) lysozyme activity, complement 3 content, liver-expressed antimicrobial peptide 2 and hepcidin, interleukin 10, transforming growth factor β1 and inhibitor of κBα mRNA levels in some intestinal segments; (3) activities and mRNA levels of copper/zinc superoxide dismutase, manganese superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferases and glutathione reductase, and NF-E2-related factor 2 (Nrf2) mRNA level in the whole intestine; (4) Claudin b, Claudin 3, Claudin c, Occludin and ZO-1 mRNA levels in some intestinal segments of grass carp. Conversely, optimal PA supplementation decreased ($P < 0.05$): (1) tumor necrosis factor α, interleukin 1β, interferon γ2, interleukin 8, nuclear factor κB P65 (NF-κB P65), IκB kinase α, IκB kinase β, IκB kinase γ and target of rapamycin (TOR) mRNA expression levels in some intestinal segments; (2) reactive oxygen species, malondialdehyde and protein carbonyl contents, and Kelch-like ECH-associating protein 1a, Kelch-like ECH-associating protein 1b in the intestine; (3) Claudin 12, Claudin 15a and myosin light-chain kinase (MLCK) mRNA levels in some intestinal segments of grass carp. In conclusion, optimum PA promoted growth, intestinal mucosal immune and physical function, as well as regulated mRNA levels of signaling molecules NF-κB P65, TOR, Nrf2 and MLCK in grass carp intestine. Based on the quadratic regression analysis of PWG and intestinal lysozyme activity, the optimal PA levels in grass carp (253.44–745.25 g) were estimated to be 37.73 mg/kg and 41.38 mg/kg diet, respectively.

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1. Introduction

The fish intestine is an important immune organ with a number of immune cells in the mucosal surface, and the intestinal health status is crucial for fish [1]. Rombout et al. [2] found that the fish intestinal health status is related to intestinal mucosal immune. Up to now, limited study has shown that nutrients could enhance intestinal mucosal immune of fish. Study from our lab showed that tryptophan could improve the intestinal mucosal immune of grass carp (*Ctenopharyngodon idella*) [3]. Pantothenic acid (PA) is a component of coenzyme A (CoA), acyl CoA and acyl carrier protein, and the coenzyme form of PA is involved in acyl group transfer reactions, tricarboxylic acid cycle and acetylation of choline [4]. However, to date, no investigation has shown the effects of PA on the intestinal mucosal immune of animals. It was proved that impaired intestinal mucosal immune resulted in a decrease of growth performance of grass carp [5]. Study from our lab also showed that PA deficiency could cause the decline of growth, digestive and absorptive abilities in Jian carp (*Cyprinus carpio var. Jian*) [6]. The intestinal digestive and absorptive abilities are largely depend on intestinal health [7]. Accordingly, there may be a possible relationship between PA and intestinal mucosal immune of animals, which warrants further investigation.

In fish, the intestinal mucosal immune largely depends on their immune function [8], which is related to antibacterial compounds like lysozyme (LA), acid phosphatase (ACP), complement and antimicrobial peptides [9,10], and cytokines like interleukin 10 (IL-10), transforming growth factor-β (TGF-β) and interferon-γ (IFN-γ) [2]. The gene expression of cytokines is regulated by intracellular nuclear factor κB (NF-κB) and target of rapamycin (TOR) signaling pathways in fish [5]. Study in human dermal fibroblasts cells showed that NF-κB inhibition reduced the expression of interleukin 8 (IL-8) [11]. Besides, TOR inhibition decreased the expression of interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) in human oral keratinocytes [12]. However, no reports at present have addressed the effects of PA on the intestinal mucosal immune by NF-κB and TOR signaling pathways in animals. In rats, it was reported that PA could activate α-Melanocyte-stimulating hormone (α-MSH) through acetylation [13]. Study has shown that α-MSH could inhibit NF-κB activation in the ileum of rats [14]. Besides, in eukaryotes, propionyl-CoA (one coenzyme of PA) is a vital part for tryptophan catabolism [15]. In our lab, study noted that tryptophan deficiency could up-regulate TOR mRNA levels in fish intestine [3]. Thus, PA may have effects on the fish intestinal mucosal immune and its possible mechanisms in fish, which is valuable for investigation.

In fish, the intestinal immune also relies on the integrity of physical barrier, which made up of intestinal epithelial cells and the tight junction (TJ) complex between epithelial cells [16]. Chen et al. [17] reported that the fish intestinal epithelial cells are particularly vulnerable to oxidative damage. To combat the oxidative damage, fish have developed antioxidant system, which includes non-enzymatic compounds (glutathione (GSH)) and enzymatic antioxidants compounds (like superoxide dismutase (SOD), catalase (CAT) and glutathione-dependent enzymes) [18]. The antioxidant enzyme activities could be a consequence of antioxidant enzymes gene expression in rats [19]. Transcriptional regulation of antioxidant enzymes gene proved to be regulated by NF-E2-related factor 2 (Nrf2) in fish [20]. However, no study has addressed the effects of PA on regulating antioxidant enzyme activities through modulating their gene transcriptions link to Nrf2 in animals. In humans, it was reported that PA participated in the formation of melatonin [21]. Study has shown that melatonin could up-regulate the gene expression of Nrf2 in the liver of hamsters [22]. Thus, these observations indicated that PA may regulate antioxidant enzyme activities through regulating their gene transcriptions, which may be related to the Nrf2 in the intestine of animals. However, the hypothesis remains to be investigated. Apart from the integrity of intestinal epithelial cells, the TJ complex between intestinal epithelial cells also plays a primary role in the intestinal physical barrier of fish [16]. The TJ complex consists of a cluster of protein species, including the transmembrane proteins Occludin and Claudins, as well as the cytoplasmatic plaque proteins zonula occludens (ZO) in humans [23]. However, there is no information about the effects of PA on the TJ proteins in animals. It was reported that PA could increase butyrate proportions in the artificial rumen [24]. In Caco-2 cells, butyrate was turned out to increase the gene expression of ZO-1 and Occludin [25]. Therefore, these data indicate a possible correlation between PA and TJ proteins in animals, which remains to be elucidated.

In worldwide, grass carp is a big contributor to the aquaculture production [26]. Currently, the PA requirement has only been determined in juvenile grass carp [27]. However, the nutrients requirements of fish may vary with different growth stage and different indices. Wilson et al. [28] reported that the PA requirement of channel catfish (*Leiocassis tenuifurcatus*) weighted at 145–155 g was higher than that of 10 g [29]. Meanwhile, in Jian carp, thiamine requirement according to the survival rate was higher [30] than that to the percent weight gain [31]. Thus, it is valuable to determine the PA requirements of grass carp based on the immune and growth indicators.

Overall, the present study was the first report to investigate the effects of PA on the intestinal mucosal immune and physical barrier of animals. In a further study, we firstly investigated the effects of PA on the mRNA expression levels of cytokines, antioxidant enzymes, TJ proteins and signal molecules like NF-κB P65, TOR and Nrf2 genes in fish intestine. The resultant findings firstly provided a partial molecular mechanism for PA regulating the intestinal mucosal immune of animals. Meanwhile, the dietary PA requirement of grass carp was also evaluated, which may provide a reference for formulating feed of grass carp.

2. Materials and methods

2.1. Experimental design and diets

The composition of the basal diet is presented in Table 1. Fish meal, casein and gelatin were used as the primary protein sources. Fish oil and soybean oil were used as the primary lipid sources. The dietary protein level was fixed at 30%, which supported the optimal growth of grass carp as described by Khan et al. [32]. The mixture of vitamin without PA was prepared according to Lin et al. [4]. Six experimental diets were obtained by supplementing the basal diet with PA at concentrations of 0 (un-supplemented control), 15.00, 30.00, 45.00, 60.00 and 75.00 mg/kg diet, and the amount of corn starch was reduced to compensate, according to the method of Wen et al. [6]. All ingredients were mixed and pelleted as previously described by Ambas et al. [33]. After being prepared completely, the diets were stored at −20 °C until use according to Wen et al. [6]. Finally, the PA concentrations in the six experimental diets were determined using high-performance liquid chromatography assay as described by Klejdus et al. [34] to be 1.31 (unsupplemented control), 15.07, 30.06, 45.09, 60.05 and 75.08 mg/kg diet.

2.2. Fish trial

Animal Care Advisory Committee of Sichuan Agricultural University specifically approved this study. Grass carp were obtained from the fisheries (Sichuan, China) and acclimatized to the experimental environment for 4 weeks, as described by Sun et al. [35]. A