

# The Effect of Allicin on Innate Immune Genes of Common Carp (*Cyprinus carpio* L)

Jean Fall (Corresponding author)

Graduate Institute of Fisheries and Aquaculture (GIFA),  
University Cheikh Anta DIOP of Dakar (UCAD), Senegal  
Tel: 221-779-564-339 E-mail: kagoshima77@yahoo.com

Mahmoud Tanekhy

Fish Diseases Dept., Faculty of Veterinary Medicine, Alexandria University, Egypt

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## Abstract

Garlic is known to possess a vast variety of biological functions. It was reported to be an antimicrobial, antithrombotic, anticancer, antioxidant and could improve the immune-system as well as had the capacity to lower serum lipid and glucose levels. The impact of allicin on signaling pathways still needs to be investigated and reports about its effect on cytokine production are inconsistent. In the present study, we investigated the influence of allicin on several carp genes and checked the immediate response of these genes. Direct p-regulation of pro-inflammatory cytokines as IL-1 $\beta$ , TNF- $\alpha$ , IL-10, TLR3, and INF- $\alpha$ , INF $\gamma$ 1 was observed after stimulation of carp head kidney (HK) cells with allicin extract. These results strongly indicate that allicin is responsible for the anti-inflammatory effects. Further, the results suggest that there are potential therapeutic effects of allicin on chronic inflammatory diseases. These effects need more investigations in aquaculture industries.

**Keywords:** Garlic, Fish, Immune genes, Diseases.

## 1. Introduction

Disease control is a major issue to the aquaculture industry; therefore it is of a great importance to prevent the outbreak of disease during the culture of commercially important fish species. One alternative way of prevention is the use of immunostimulants that affect the host rather than the pathogen, elevating nonspecific and specific host defenses.

Garlic's historic and worldwide medicinal uses have made it one of the most extensively studied medicinal herbs. However, the actual curative benefits of this member of the

Liliaceae family are unclear. Garlic is considered safe by the food drug administration (FDA), based on the lack of known serious adverse outcomes despite culinary and medicinal use throughout human history. The main purported active ingredient, allicin, is degraded by crushing, heat, and acid; thus, efficacy is optimized by consuming raw cloves or enteric-coated tablets. The usual dose is 300 mg, taken 2 to 3 times per day, standardized to at least 1.3% allicin (equivalent to approximately 3 g or 1 fresh clove daily). The enzyme allinase converts alliin to allicin (diallyl thiosulphinate), which is an extremely unstable compound that is responsible for the pungent smell of garlic (Block, 1985). It becomes quickly decomposed into organo-sulphur compounds, i.e. diallyl sulphide (DAS), diallyl disulphides (DADS), polysulphides and ajoenes (Miron et al., 2004). S-allyl cysteine is a relatively stable, odorless, bioavailable and water-soluble compound and is considered to contribute to the pharmacological and immunological activities of garlic (Corzo-Martinez et al., 2007). Allicin diallyl thiosulphinate is an active but volatile compound of garlic. It has been implicated in antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria, and has antiviral, antifungal and anti-protozoal activity (Rose et al., 2005). It has been found that the antioxidant allicin scavenges OH and inhibits lipid peroxidation. Higher doses (>100 µM) of allicin have proven toxic to mammalian cells (Hasan et al., 2007). Moreover, Shahsavani et al. (2011) showed that allicin supplementation is effective in decreasing lead accumulation in all examined tissues of common carp. The promising ameliorative effects of allicin on tissue lead levels of common carp make it a good candidate for therapeutic intervention of lead poisoning. Miron et al. (2000) concluded that allicin could easily diffuse into the internal volume of vesicles or into the cytoplasm of red blood cells. Allicin have the ability in preventing immune mediated concanavalin A-induced liver damage in mice (Santhosha et al., 2013). Allicin modulates the effect of T-cells and adhesion molecules and exerts an inhibitory effect on NF-kappa B activation and hence prevents liver damage. Studies have also suggested that allicin could be used therapeutically in treating chronic inflammatory diseases like inflammatory bowel disease.

Cytokines are small glycoproteins involved in extracellular signaling and play a significant role in balancing host immune responses. Based on their structural features, cytokines have been grouped into several families, including Interleukins (IL) such as IL-1, -2, -6, -12, -10, -17, Tumor necrosis factors (TNFs), transforming growth factors, and chemokines (Vilcek, 2003). These molecules regulate local and systemic immune inflammatory and regulatory events. The differentiation of Th cells into Th1–Th2 cells is important as hosts mount effective immune responses. The differentiation and balance between Th1–Th2 type response is predominately mediated by the respective cytokines. Previously, identification of fish cytokines was based mainly on EST- and PCR-mediated homology cloning using degenerate PCR primers (Savan & Sakai, 2006).

Several fish cytokine genes have been isolated and characterized in recent years, and researchers have used mRNA expression as a tool for measuring immune responses (genomic database of zebrafish and fugu). Although not all genes discovered by homology cloning may encode for proteins with the same function as the query genes, it appears that fish have most of the cytokines relevant to responses to bacteria, viruses, inflammation, or cell proliferation and chemo-attraction. In particular, pro-inflammatory cytokines, including IL-1 $\beta$ , TNF- $\alpha$ ,

IL-8, IL-12, INFs, and IL-10 are commonly used immune-regulatory genes in fish (Castillo et al., 2009; Tanekhy et al., 2010).

The present study was carried out to investigate the effect of allicin, a crude garlic extract on the immune related genes expressions in the common carp, *Cyprinus carpio* L.

## 2. Material and Methods

### 2.1 Fish

Adult common carp (mean mass 300g) were obtained from Susano farm, Miyazaki, Japan. They were checked thoroughly for injury and disease conditions, and only healthy fishes were used for this study. After washing with 0.01% KMnO<sub>4</sub> solution for 15 min, they were placed in nine plastic pools (500 L) containing non-chlorinated water. Prior to the start of the experiment, the fishes were acclimatized to the food and laboratory conditions with 12 h dark and 12 h light cycles, pH range of 6.95 to 7.60 and temperature ranging from 16 to 24 °C for 15 days.

### 2.2 Allicin (Garlic extract)

Fresh garlic cloves (70 g) were blended in 35 ml distilled H<sub>2</sub>O, centrifuged, ultra-filtered through a 10 kDa cut-off membrane (Flowgen, UK) and sterilized by filtration (0.45 μm). By subtracting the weight of the insoluble material from the weight of the original cloves, the final concentration of the garlic extract in solution was determined to be 57.1% (w/v). Aliquots were stored at -20 °C until required.

### 2.3 In vitro Stimulation Using Allicin

A preliminary in vitro stimulation test using different concentrations of allicin was conducted. The in vitro stimulation test was done by using concentration of 6 μg/μl, 12 μg/μl, 25 μg/μl and 50 μg/μl of allicin at 4h post-stimulation. The lower concentration of allicin 6 μg/μl resulted in a significant increase of all immune genes used in this study. However, higher concentrations of 25 and 50 μg/μl resulted in a marked decrease in these genes (unpublished data). Therefore, we decided to use the concentration of 6 μg/μl of allicin for the gene expression study.

The head kidney (HK) cells of five fish were isolated according to the modified method described by Braun-Nesje et al (1982). The cells were removed from the fish and filtered through a nylon mesh with RPMI 1640 medium (Nissui, Japan) containing 1 % streptomycin / penicillin (S/P, Gibco, USA), 0.2% heparin (Sigma, USA) and 10 % carp serum (CS). HK cells of carp were evenly dispersed in the RPMI 1640 medium and stimulated with 6 μg/μl of allicin at 0h (control), 1h, 4h, 8h, and 12 h post-stimulation at 22 °C, in RPMI 1640 medium supplemented with 50 μg / ml of ampicillin.

### 2.4 Expression Analysis of Carp Cytokine Genes

#### 2.4.1 RT-PCR

Total RNA was isolated from treated Carp HK cells using ISOGEN (Nippon Gene, Osaka, Japan) according to the manufacturer's instructions. cDNA synthesis was then performed using ReverTra Dash (Toyobo, Osaka, Japan). Using the synthesized cDNA as a template, PCR reactions were performed according to the following protocol: 1 μL of cDNA was mixed with 5 μL of dNTPs (10 μM of each dNTP) and 10× Gene Taq Universal buffer, 0.5

$\mu$ L of *Taq* polymerase (5 units/ $\mu$ L), 5  $\mu$ L of each specific primer (5  $\mu$ M) and 28.5  $\mu$ L of distilled water. Specific carp  $\beta$ -actin primers served as internal control. Expression levels of the carp interleukin (IL)-1 $\beta$  (AB010701; Fujiki et al., 2000), IL-10 (AB110780; Savan et al., 2003), type1 INF (Kitao et al, 2009), INF $\gamma$ 1, INF $\gamma$ 2, TLR3 and TNF - $\alpha$  (AB112424; Saeij et al., 2003) have been performed. The sequences, annealing temperature and PCR product size for each of the specific primer pairs for carp  $\beta$ -actin and the cytokines observed in this study are outlined in Table 1.

Table 1. Common carp immune related genes primers and the PCR conditions with accession numbers

Primers	Sequence (5'-3')	Cycle No	An. Temp (°C)	Amplicon size (bp)	Accession No.
INF- $\alpha$ F INF- $\alpha$ R	TGCATATGGCTCGGCCAATA GTCAAGACAAGAAACCTCACC	35	61	804	AB376667
IL-1 $\beta$ F IL-1 $\beta$ R	GGAGAATGTGATCGAAGAGC GTAGAGGTTGCTGTTGGA	30	61	280	AJ245636
IL-10 F IL-10 R	TGATGACATGGAACCATTACTGG CACCTTTTCCTTCATCTTTTCA	30	60	284	AB110780
TNF- $\alpha$ F TNF- $\alpha$ R	GCTGTCTGCTTCACGCTC AAAGCCTGGTCCTGGTTC	30	58	188	AJ311800
INF $\gamma$ 1 F INF $\gamma$ 1 R	GTCGCTGCTGCTTGATAGAA CTGAAGCTCCCTCCATACTT	32	60	166	AM261214
INF $\gamma$ 2 F INF $\gamma$ 2 R	GAGGAACCTGAGCAGAATCT CCTTGATCGCCCATAGTGTT	32	60	202	AM168523
TLR3 F TLR3 R	GCAGACAACAACCTTGGCTA CATGCAGCTGATCCAAGAGA	32	59	197	DQ885910
$\beta$ -actin F $\beta$ -actin R	ACCTCATGAAGATCCTGACC TGCTAATCCACATCTGCTGG	24	60	312	M24113

PCR products were electrophoresed on a 1.5 % agarose gel to detect the specific gene bands.

### 2.5 Semi-Quantitative Analysis of RT-PCR Products

Semi-quantitative analysis was undertaken in accordance to the method described by Kono et al. (2004). The carp cytokine /  $\beta$ -actin gene ratio and shrimp genes / EF ratio were determined by densitometry, undertaken by measuring photo-stimulated luminescence values using SCIENCE LAB99 IMAGE GAUGE software (Fujifilm, Tokyo, Japan), and by comparing the transcript levels of these genes to the internal genes.

### 2.6 Statistical Analysis

Data is expressed as mean  $\pm$  SEM. PCR results for the infected and control groups were analyzed using ANOVA and Tukey's significant difference tests.

### 3. Results and Discussion

Some adjuvants and immunostimulant substances such as azadirachtin, chitin, CpG-ODNs, sodium alginate and iota-carrageenan, yeast,  $\beta$ -glucans as well as bacterial polysaccharides are commonly used to prevent occurrence of disease and to enhance immunity of fish.  $\beta$ -glucan administration has been reported to increase antibody production, complement activity, lysozyme activity, macrophage activity and respiratory burst of channel catfish *Ictalurus punctatus*, rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar*, and gilthead seabream *Sparus auratus*. It is demonstrated that dietary garlic or garlic extract allicin enhance the non-specific immune mechanism of rainbow trout *Oncorhynchus mykiss* by stimulating the proliferation of immune cells, and enhancing phagocytosis, oxidative burst and lysozyme activities, Nya and Austin (2009, 2010) and Agatha A. Nwabueze (2012).

The pro-inflammatory cytokine (TNF- $\alpha$ ) is key components in innate immunity and the inflammatory response in fish (Saeij et al., 2003). TNF- $\alpha$  is also an important macrophage-activating factor (MAF) produced by leukocytes. It is synthesized by various cell types upon stimulation with endotoxin, inflammatory mediators, or cytokines such as IL-1 $\beta$ , and in an autocrine manner, upon stimulation by TNF- $\alpha$  itself (Hirono et al., 2000). Expression studies, particularly those conducted in vitro, have generally demonstrated that TNF- $\alpha$  mRNA expression and regulation in trout, carp, and flounder is similar to that observed in mammals, with similar activation kinetics (Wang & Secombes, 2013). In the present study, the expression of TNF- $\alpha$  was significantly increased 1 and 4 hours post stimulation as compared to the control group, the highest expression of the IL-10 gene was observed 4 hours post stimulation (Figure 1).

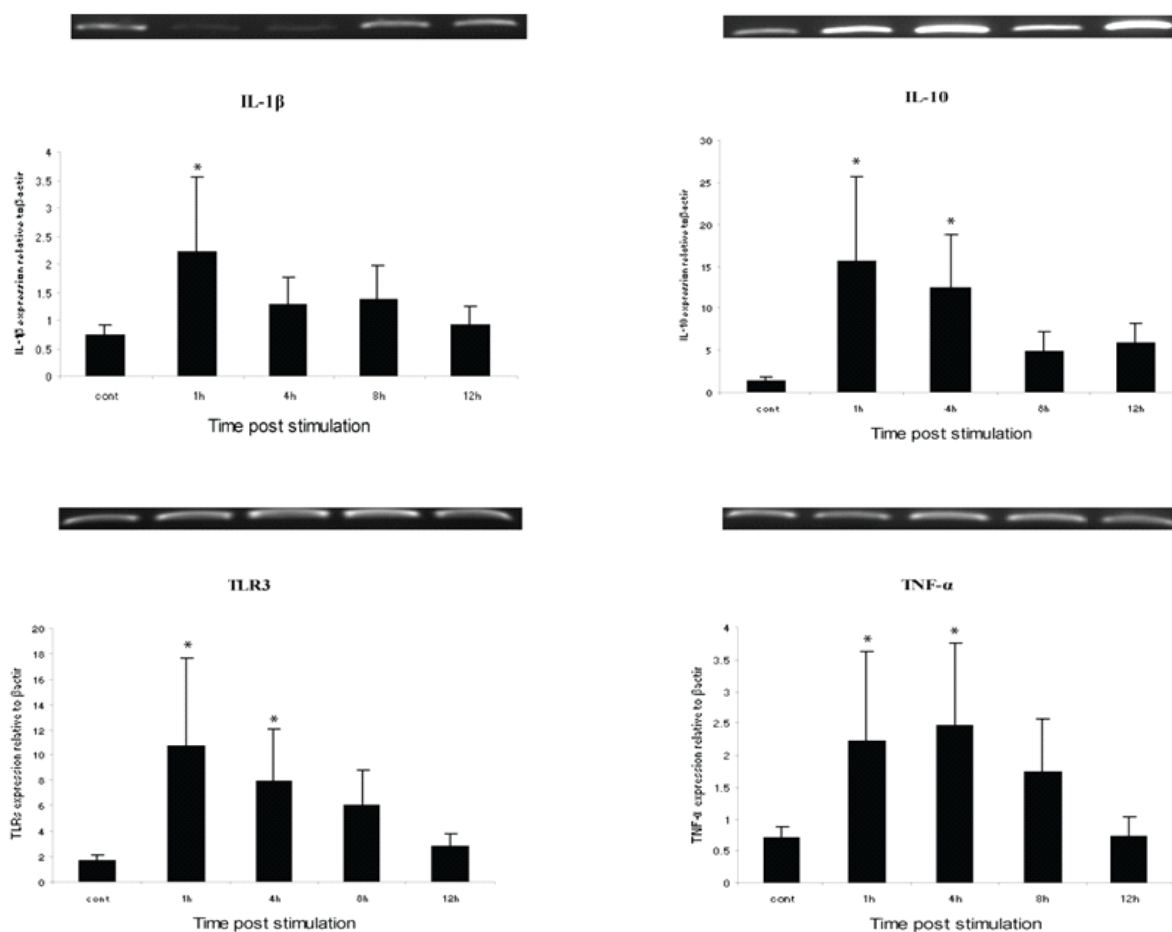


Figure 1. Expression analysis of IL-1, IL-10, TLR3 and TNF $\alpha$  in kidney of *Cyprinus carpio* after being stimulated with crude garlic extract

Garlic has been shown to have anti-inflammatory and pro-apoptotic properties among others. It was hypothesized that treating placental explants with garlic may inhibit the production of inflammatory cytokines tumor necrosis factor (TNF- $\alpha$ ). A reduction in TNF $\alpha$  production was evident at both low (10  $\mu$ g/mL) and high (500  $\mu$ g/mL and 1000  $\mu$ g/mL) garlic concentrations in preeclamptic explants (Markis et al., 2004). TNF- $\alpha$  is an important pro-inflammatory cytokine. It is reported that allicin markedly inhibited the spontaneous TNF- $\alpha$  expression and TNF- $\alpha$  -induced secretion of IL-1 $\beta$ , from the two different cell lines in a dose-dependent manner and suppressed the expression of IL-1 $\beta$  mRNA level (Lang et al., 2002).

IL-1 $\beta$  is produced by macrophages, monocytes, and dendritic cells. IL-1 $\beta$  acting as an important mediator of the inflammatory response and is involved in a variety of cellular activities (Miliani et al., 2007). The expression of IL-1 $\beta$  was found to be higher than the controls 1h post stimulation with garlic extract (Figure 1) giving onset up-regulation. This activation of IL-1 $\beta$  maybe related with the enhancement of macrophage functions. Fujiki et al. (1999, 2000) analyzed the expressed genes in HK of carp injected with sodium alginate and scleroglucan by suppression subtractive hybridization and reported the expression of immune-related genes like IL-1 $\beta$ . Kono et al. (2002) demonstrated the activation of IL-1 $\beta$  in

carp injected with PG. IL-1 $\beta$  expression in rainbow trout HK leucocytes is induced by LPS stimulation (Zou et al., 2000). Jørgensen et al. (2001) showed that CpG ODN 1668 activates trout macrophages to express IL-1 $\beta$ . CpG ODN 1668, in concentrations varying from 2 to 20  $\mu$ M, also produced significant induction of IL-1 $\beta$  expression.

IL-10 is an immune-regulatory cytokine that influences the immunological system, both on the innate and cell-mediated response. The up-regulation of IL-10 was recorded at 1 and 4h compared to the control group. The highest expression of the IL-10 gene was observed at 1h (Fig.1). IL-10 expression has been identified in carp after LPS stimulation to the HK (Tanekhy et al., 2009). It was hypothesized that treating placental explants with garlic may stimulate the production of anti-inflammatory cytokines IL-10 by the placental explants. The lower concentration of garlic 10  $\mu$ g/mL resulted in a significant increase in IL-10 production by normal placentas. However, higher concentrations of 500  $\mu$ g/mL and 1000  $\mu$ g/mL resulted in a marked decrease in IL-10 production (Markis et al., 2004). In the current study, the expression of the IL-10 gene was significantly increased at 1 and 4h stimulation with allicin. IL-10 is generally known as an immunosuppressive cytokine. This is because, in rainbow trout and carp, IL-10, like other inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$  is expressed soon after LPS stimulation, whereas in humans, IL-10 is not expressed so quickly (Inoue et al., 2005).

Toll-like receptors (TLRs) play an essential role in the innate immune system by initiating and directing immune responses to pathogens and control activation of adaptive immune responses. TLR3 triggers specific signaling pathways that culminate in the activation of NF-kB and IRF3 transcription factors, as well as apoptosis, enabling the host to mount an effective innate immune response through the induction of cytokines, chemokines, and other pro-inflammatory mediators.

In the present study, the expression of TLR3 was significantly increased 1 and 4h post stimulation as compared to the control group. The highest expression of the IL-10 gene was observed at 1h (Figure 1). Poly I:C has been shown to bind TLR3. TLR3 deficient mice macrophages were shown to have greatly reduced responses to poly I:C. Human natural killer cells (NK cells) have been reported to express TLR3, to up-regulate TLR3 mRNA and cytotoxic activity following poly I:C stimulation.

Interferon (IFN) is a protein that is secreted from cells in response to invasion by pathogens, and is classified into type-I IFN (I-IFN) and type-II (II-IFN). IFN are produced in numerous cell types including lymphocytes (T and B cells), macrophages, fibroblasts, blood vessel endothelial cells and osteogenetic cells, and are known to be important elements in antiviral response (Samuel, 2001). In the present study, the expression of INF- $\alpha$  was significantly increased at 1 and 4h compared to the control group. The highest expression of the INF- $\alpha$  gene was observed at 1h (Figure 2).

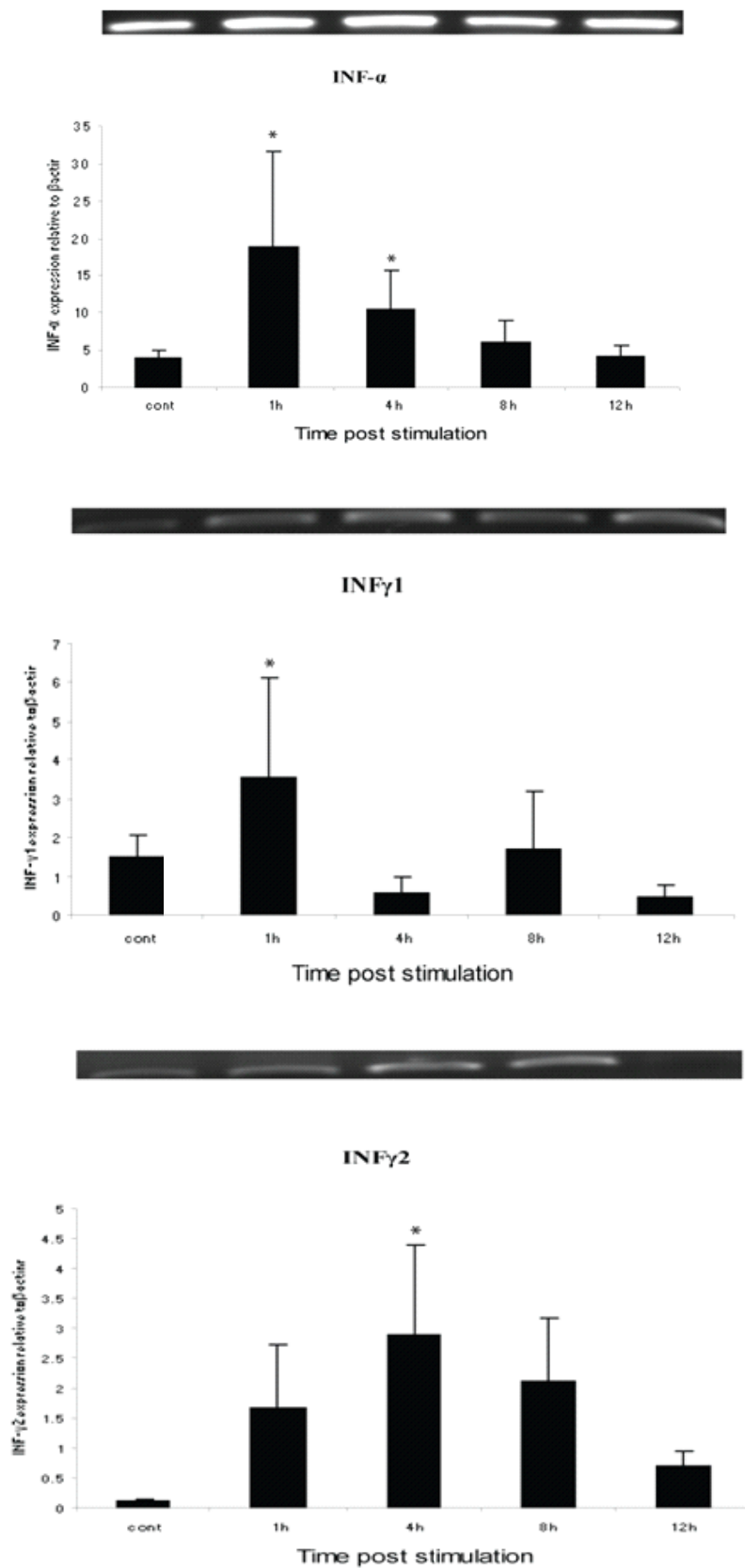


Figure 2. Expression analysis of interferons (INF $\alpha$ , INF $\gamma$ 1 and INF $\gamma$ 2) in kidney of *Cyprinus carpio* after being stimulated with crude garlic extract



The expression of INF $\gamma$ 1 and was INF $\gamma$ 2 was significantly higher than the control group at 1h (Figure 2). The CpG ODN 1668 used has been shown by several different groups to stimulate immune mechanisms in higher vertebrates and recently it was reported to activate Atlantic salmon leucocytes to produce IFN-like cytokines (Jørgensen et al., 2001). There was no effect of different types of stimulants of IFN production, including LPS, poly I:C and the mitogen PHA, on the inducibility of the cell line in rainbow trout (Castro et al., 2010). PHA is a mitogen widely used to induce blastogenesis and mitosis in peripheral lymphocytes and to induce IFN-gamma expression. It was shown previously to strongly induce trout IFN-gamma expression in primary cultures of head kidney leucocytes at low doses (10 mg/ml). Using the same dose, Castro et al. (2010) have also detected a correlated expression in the primary cell line cultures after 4 h of PHA stimulation as an IFN-gamma induced gene. Recently, PHA was described as an activator for type I IFNs in RTG-2 cells using high concentrations of the lectin (50 mg/ml).

In conclusion, the present study documented that allicin activate both non-specific humoral immunity and non-specific cellular immune ability by increasing expression of cytokine genes in head kidney of carp. The potent immunostimulatory activities of allicin reported for fish in this study suggest allicin as an interesting immunostimulant to be tested in fish vaccines challenge test with pathogens.

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#### **References**

- Agatha, A. N. (2012). The Effect of Garlic (*Allium sativum*) on Growth and Haematological Parameters of *Clarias gariepinus* (Burchell, 1822). *Sustainable Agriculture Research*, 1(2).
- Braun-Nesje, R., Kaplan, G., Sejelid, R. 1982. Rainbow trout macrophages in vitro: Morphology and phagocytic activity. *Dev. Comp Immunol*, 6, 281-291. [http://dx.doi.org/10.1016/S0145-305X\(82\)80011-6](http://dx.doi.org/10.1016/S0145-305X(82)80011-6)
- Bicknell, R. A. Salmo. (2005). The use of immunostimulants in fish larval aquaculture. *Fish Shellfish Immunol*, 19, 457-472. <http://dx.doi.org/10.1016/j.fsi.2005.03.008>
- Castillo, J., Tales, M., Mackenzie, S., Tort, L. 2009. Stress-related hormones modulate cytokine expression in the head kidney of gilthead Seabream (*Sparus aurata*). *Fish shellfish Immunol*, 2, 493-499. <http://dx.doi.org/10.1016/j.fsi.2009.06.021>
- Castro, R., Martin, S. A. M., Zou, J., & Secombes, C. J. (2010). Establishment of an IFN-g specific reporter cell line in fish. *Fish shellfish Immunol*, 28, 312-319. <http://dx.doi.org/10.1016/j.fsi.2009.11.010>
- Cavalitto, C., & Bailey, J. H. (1944). Allicin, the antibacterial principle of *Allium sativum*. Isolation, physical properties and antibacterial action. *J AM Chem Soc*, 66. <http://dx.doi.org/10.1021/ja01239a048>
- Celiini, L., Di Campli, B., Masulli, M., Di Bartolomeo, S., & Aliocati, N. (1996). Inhibition of *Helicobacter pylori* by garlic extract (*Allium sativum*). *FEM Immenol Med Micrbiol*, 13, 273-277. <http://dx.doi.org/10.1111/j.1574-695X.1996.tb00251.x>

- Corzo-Martinez, M., Corzo, N., & Villamiel, M. (2007). Biological properties of onions and garlic. *Trends Food Sci Techn*, 18, 609-625. <http://dx.doi.org/10.1016/j.tifs.2007.07.011>
- Elmore, G. S., & Feldberg, R. S. (1994). Alliin lyase localization in bundle sheaths of garlic clove (*Allium sativum*). *Am J Bot*, 81, 89-94. <http://dx.doi.org/10.2307/2445567>
- Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S., & Girardin, S. E. (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1 $\beta$  generation. *Clin Exp Immunol*, 147(2), 227-235.
- Fujiki, K., Shin, D. H., Nakao, M., & Yano, T. (2000). Molecular cloning and expression analysis of carp (*Cyprinus carpio*) interleukin-1 beta, high affinity immunoglobulin E Fc receptor gamma subunit and serum amyloid A. *Fish shellfish Immunol*, 10(3), 229-42. <http://dx.doi.org/10.1006/fsim.1999.0253>
- Gonzalez-Fandoz, F., Garcia-Lopez, M., Sierra, M., & Otero, A. (1994). Staphylococcal growth and enterotoxins (A-D) and thermonuclease synthesis in the presence of dehydrated garlic. *J Appl Bacteriol*, 77, 549-552. <http://dx.doi.org/10.1111/j.1365-2672.1994.tb04400.x>
- Hirono, I., Nam, B. H., Kurobe, T., & Aoki, T. (2000). Molecular cloning, characterization, and expression of TNF cDNA and gene from Japanese flounder *Paralichthys olivaceus*. *J Immunol*, 165(8), 4423-7. <http://dx.doi.org/10.4049/jimmunol.165.8.4423>
- Huges, E. G., & Lawson, L. D. (1991). Antimicrobial effects of *Allium sativum* L. (garlic) *Allium amelopratrum* (elephant garlic) and *Allium cepa* (onion) garlic compounds and commercial grade garlic supplements. *Phytother Res*, 5, 154-158. <http://dx.doi.org/10.1002/ptr.2650050403>
- Inoue, Y., Kamota, S., Ito, K., Yoshiura, Y., Ototake, M., Moritomo, T., & Nakanishi, T. (2005). Molecular cloning and expression analysis of rainbow trout (*Oncorhynchus mykiss*) interleukin-10 cDNAs. *Fish Shellfish Immunol*, 18, 335-344. <http://dx.doi.org/10.1016/j.fsi.2004.08.004>
- Jørgensen, J. B., J. Zou, A. Johansen, C., & Secombes, J. (2001). Immunostimulatory CpG oligodeoxynucleotides stimulate expression of IL-1beta and interferon-like cytokines in rainbow trout macrophages via a chloroquine-sensitive mechanism. *Fish Shellfish Immunol*, 11, 673-682. <http://dx.doi.org/10.1006/fsim.2001.0344>
- Koch, H. P., & Lawson, L. D. (1996). Garlic, the science and therapeutic application of *Allium sativum* L. and related species. In D. C. Retford (Ed), *Williams and Wilkins, Baltimore* (pp. 1-233).
- Kitao, Y., Kono, T., Korenaga, H., Iizasa, T., Nakamura, K., Savan, R., & Sakai, M. (2009). Characterization and expression analysis of type I interferon in common carp, *Cyprinus carpio* L. *Mol Immunol*, 46, 2548-2556. <http://dx.doi.org/10.1016/j.molimm.2009.05.012>
- Kono, T., Ponpornpisit, A., & Sakai, M. (2004). The analysis of expressed genes in head kidney of common carp, *Cyprinus carpio* L. stimulated with peptidoglycan. *Aquaculture*, 235, 37-52. [http://dx.doi.org/10.1016/S0044-8486\(03\)00447-2](http://dx.doi.org/10.1016/S0044-8486(03)00447-2)
- Lang, R., D. Patel, J. J., Morris, R. L., & Rutschman, P. J. (2002). Shaping gene expression in activated and resting primary macrophages by IL-10. *J Immunol*, 169, 2253-2263. <http://dx.doi.org/10.4049/jimmunol.169.5.2253>

- Makris, C. E., Thornton, B., & Xu, A. (2004). Garlic increases IL-10 and inhibits TNF $\alpha$  and IL-6 production in endotoxin-stimulated human placental explants. *Placenta*, 26(10), 828-834. <http://dx.doi.org/10.1016/j.placenta.2004.10.019>
- Miron, T., Rabinkov, A., Mirelman, D., Wilchek, M., & Weiner, L. (2000). The mode of action of allicin: Its ready permeability through phospholipid membranes may contribute to its biological activity. *Biochim Biophys Acta*, 1463, 20-30. [http://dx.doi.org/10.1016/S0005-2736\(99\)00174-1](http://dx.doi.org/10.1016/S0005-2736(99)00174-1)
- Miron, T., Bercovici, T., Rabinkov, A., Wilchek, M., & Mirelman, D. (2004). allicin: preparation and applications. *Anal Biochem*, 331, 364-369. <http://dx.doi.org/10.1016/j.ab.2004.03.054>
- Nya, E. J., & Austin, B. (2009). Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis*, 32, 963-970. <http://dx.doi.org/10.1111/j.1365-2761.2009.01101.x>
- Rabinkov, A., Miron, T., Konsrantinovski, L., Wilchek, M., Mirelman, D., & Weiner, L. (1998). The mode of action of allicin: Trapping of radicals and interaction with thiol containing proteins. *Biochim Biophys Acta*, 1379, 233-244. [http://dx.doi.org/10.1016/S0304-4165\(97\)00104-9](http://dx.doi.org/10.1016/S0304-4165(97)00104-9)
- Rose, P., Whiteman, M., Moore, P. K., & Zhu, Y. Z. (2005). Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus *Allium*. The chemistry of potential therapeutic agents. *Natural Product Reports*, 22, 351-368. <http://dx.doi.org/10.1039/b417639c>
- Savan, R., Igawa, D., & Sakai, M. (2003). Cloning, characterization and expression analysis of interleukin10 from the common carp, *Cyprinus carpio* L. *Eur J Biochem*, 270(23), 4647-4654. <http://dx.doi.org/10.1046/j.1432-1033.2003.03854.x>
- Savan, R., & Sakai, M. (2006). Genomics of fish cytokines. *Comp Bioch Physiol*, 1, 89-101. <http://dx.doi.org/10.1016/j.cbd.2005.08.005>
- Samuel, C. E. (2001). Antiviral actions of interferons. *Clin Microbiol Rev*, 14(4), 778-809. <http://dx.doi.org/10.1128/CMR.14.4.778-809.2001>
- Saeij, J. P., Stet, R. J., de Vries, B. J., van Muiswinkel, W. B., & Wiegertjes, G. F. (2003). Molecular and functional characterization of carp TNF: A link between TNF polymorphism and trypanotolerance? *Dev Comp Immunol*, 27(1), 29-41. [http://dx.doi.org/10.1016/S0145-305X\(02\)00064-2](http://dx.doi.org/10.1016/S0145-305X(02)00064-2)
- Shahsavani, D., Baghshani, H., & Alishahi, E. (2011). Efficacy of allicin in decreasing lead (Pb) accumulation in selected tissues of lead-exposed common carp (*Cyprinus carpio*). *Biol Trace Elem Res*, 142, 572-580. <http://dx.doi.org/10.1007/s12011-010-8801-3>
- Santhosha, S. G., Prakash, J., & Prabhavathin, S. N. (2013). Bioactive components of garlic and their physiological role in health maintenance: A review. *Food Bioscience*, 3, 59-74. <http://dx.doi.org/10.1016/j.fbio.2013.07.001>
- Tanekhy, M., Kono, T., & Sakai, M. (2009). Expression profile of cytokine genes in the common carp species *Cyprinus Carpio* L. following infection with *Aeromonas hydrophila*. *Bull Eur Ass Fish Pathol*, 29, 197-203.
- Tanekhy, M., Kono, T., & Sakai, M. (2010). Cloning, characterization, and expression

analysis of Toll-like receptor-7 cDNA from common carp, *Cyprinus carpio* L. *Comp Biochem Physiol Part D*, 5, 245-255. <http://dx.doi.org/10.1016/j.cbd.2010.07.001>

Yamada, Y., & Azuma, K. (1997). Evaluation of the *in vitro* anti-fungal activity of allicin. *Antimicrob Agents Chemothe*, 1, 743-749.

Vilcek, J. (2003). Fifty years of interferon research: aiming at a moving target. *Immunity*, 25, 343-8. <http://dx.doi.org/10.1016/j.immuni.2006.08.008>

Wang, T., & Secombes, C. J. (2013). The cytokine networks of adaptive immunity in fish. *Fish Shellfish Immunol*, 35(6), 1703-1718. <http://dx.doi.org/10.1016/j.fsi.2013.08.030>

Zou, J., Peddie, S., Scapigliati, G., Zhang, Y., Bols, N. C., Ellis, A. E., & Secombes, C. J. (2003). Functional characterization of rTNF in rainbow trout. *Onchorhyncus mykiss*. *Dev Comp Immunol*, 27, 813-822. [http://dx.doi.org/10.1016/S0145-305X\(03\)00077-6](http://dx.doi.org/10.1016/S0145-305X(03)00077-6)

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