Cao Y, et al., J Agron Agri Sci 2024, 7: 054
DOI: 10.24966/AAS-8292/100054

HSOA Journal of Agronomy & Agricultural Science

Research Article

Alternation of immune function and gut microbiota of Koi carp in response to dietary garlic powder

Cao Y[1]*, Du Y[1], Zhang R[2] and Zhao H[1]

[1]*Animal Science and Technology College, Beijing University of Agriculture, Beijing 102206, China*

[2]*Beijing Key Laboratory of Fishery Biotechnology, Fisheries Science Institute, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100068, China*

Abstract

This research aimed to assess the effects of garlic powder (GAL) on immune function, intestinal histology, and bacterial community in koi carp. Fish were randomly assigned to four treatments and fed diets which contained various concentrations of GAL at 0 (control), 500 (GAL-L), 1,500 (GAL-M) or 4,500 (GAL-H) mg/kg diet. After the 8-w feeding experiment, blood was sampled to analyze serum parameters. Gut samples were collected to determine intestinal morphology and microbiota. The result showed that dietary GAL reduced the levels of serum TNF-α, IL-1β and IL-6 ($P=0.0001$; $P<0.0001$; $P<0.0001$), but increased the activities of lysozyme, complement C3 and C4 ($P=0.0008$; $P<0.0001$; $P<0.0001$). Serum SOD and GPx activities were higher in the GAL-M and GAL-H groups ($P=0.0488$; $P<0.0001$), while MDA activities was weaker in the GAL-L and GAL-H groups than in the control group ($P=0.0004$). Dietary GAL increased intestinal muscle thickness in the GAL-L and GAL-M groups ($P=0.0028$). Additionally, the gut microbiota of the GAL-M group was different from that of the control ($P=0.007$). 28 differentially abundant taxa were identified as potential biomarkers by LEfSe analysis. Collectively, GAL-meditated benefits may be associated with intestinal microbiota

Keywords: Antioxidant; Anti-inflammatory; Gut microbiota; Intestinal morphology; Immune response

***Corresponding author:** Cao Y, Animal Science and Technology College, Beijing University of Agriculture, Beijing 102206, China. E-mail: caoyongchun001bn@sina.com

Citation: Cao Y, Du Y, Zhang R, Zhao H (2024) Alternation of immune function and gut microbiota of Koi carp in response to dietary garlic powder. J Agron Agri Sci 7: 054.

Received: December 26, 2023; **Accepted:** January 09, 2024; **Published:** January 16, 2024

Introduction

Since the 1990s, koi breeding has become an important economic industry in China's fisheries. However, large-scale and high-density farming has led to a decline in the disease resistance of koi [1]. The increase in the frequency of diseases can cause damage to the body surface, resulting in scarring, which seriously reduces the ornamental quality of Koi and poses a major threat to the sustainable development of the Koi industry. In order to control diseases of aquatic organisms, breeders use a large number of compounds and antibiotics as growth promoters, which can harm the fish and may even cause illness in humans [2].

Some plant derivatives, which, after a series of extractions or processing, promote growth in fish, are also used as immunostimulants in fish diets. Many plant extracts are now used as aquafeed additives and are safe and effective during feeding [3]. For example, thyme, carvacrol, and limonene have been proven to have a beneficial impact on the nutrition and health of animals [4]. Garlic is part of the genus Allium in the lily family and contains allicin and other bioactive compounds. It originated in the Mediterranean Sea and is now cultivated worldwide, mainly in China, Korea, and the United States, among other countries. Since the beginning of its cultivation, garlic has been studied for its flavoring and medicinal properties [5]. Garlic is pharmacologically active because it contains a complex chemical composition that includes proteins, flavonoids, macronutrients, oligosaccharides, and other substances in addition to sulfur-containing organic compounds. Currently, garlic is generally used in aquaculture by oral administration, injection, and infusion, with oral administration being the most frequently used method [6].

According to research studies, garlic has a variety of health benefits, including broad-spectrum antibacterial, sterilization [7] anti-inflammatory, and immunomodulation [8]. Garlic can be added in a variety of forms, but it can all be added to feed as an alternative to antibiotics [9]. Garlic powder has not been studied in koi, so the focus of this experiment was to assess whether garlic powder (GAL) produced beneficial effects on immune function, gut histology, and bacterial communities in koi.

Materials and methods

Fish and experimental design: The experiments were carried out at the Beijing Fisheries Research Institute of Beijing Academy of Agriculture and Forestry Sciences, China. 180 fish (15.2 ± 2.88 g) were purchased from a commercial fish farm and first fed in the culture system for 4 weeks to acclimatise to the environment. Fish were assigned to 4 treatments with 3 replications for 56 days (n= 45 fish per treatment). The recirculating rearing system contains three 200-L tanks per treatment. The inclusion rates of garlic powder (25% purity, *Allium sativum L.*) in the feed are 0 (Control), 500 (GAL-L), 1,500 (GAL-M) and 4,500 (GAL-H) mg/kg. Experimental fish were fed at 9am and at 5pm. The fish were weighed individually before starting to feed and 2% of their body weight was calculated as the amount to be fed. The composition and nutritional content of the basal diets for

the experimental fish are shown in Table 1. During the experimental period, water was exchanged daily and the water condition was maintained as follows: temperature 21±1°C, dissolved oxygen 8.0-8.5 mg/L, ammonium 0-1.0 mg/L, and nitrite 0-0.05 mg/L.

Ingredients	g/kg
Soybean meal	300
Steam dried fish meal	317
Wheat flour	200
Wheat bran	150
Fish oil	23
Trace minerals and vitamins[1]	10
Nutrient	g/kg
Dry Matter	896
Crude Protein	401.1
Crude Fat	62.9
Crude Ash	84.1
Calcium	13.1
Total Phosphate	10.9
Salt	11.1

Table 1: Ingredients and nutrient profiles of the basal feed.

Sampling: After 56 days of rearing experiments, the fish were anesthetized by immersion in 100 mg/L MS-222 (n=9 fish per treatment). The anaesthetised fish is placed on an ice box and a vein is found in the tail for blood sampling. Blood samples were left to stand for 4 hours in a refrigerated chill, then centrifuged at 1200 × g for 10 min and the serum was decanted to analyze the concentrations of tumor necrosis factor α (TNF-α), interleukin (IL-)1β, interleukin (IL-)6, lysozyme, complement C3, C4, Superoxide Dismutase Activity (SOD), Glutathione Peroxidase (GPx) and Malondialdehyde (MDA). The fish were subsequently dissected and the entire intestinal tracts were carefully sampled. Two segments of the proximal intestine (approximately 0.5 cm long) were collected. One segment was immobilized in 10% neutral formalin liquid for the evaluation of intestinal morphology, the other segment was frozen at −20°C for the measurement of the bacterial community.

Measurements

Blood parameters: Measurement of TNF-α, IL-1β and IL-6 levels in fish serum, this experiment was performed using an Enzyme-Linked Immunosorbent Assay (ELISA). Determination of the absorbance of a suspension of Micrococcus lysogenicus at 450 nm as a means of determining the lysozyme activity [10]. Complement C3 or C4 activity was determined by detecting its increase in absorbance after immunity response of C3 or C4 and its increased antibody [11]. The activity of SOD was measured based on its ability to restrain the autoxidation process of pyrogallol [12]. GPx activity was assayed using a 5,5′-dithiobis (2-nitrobenzoic acid) reagent as per the method of Ellman [13]. The concentration of MDA was determined using Thiobarbituric Acid (TBA) as per the method of Buege and Aust [14].

Intestinal morphology: Intestinal samples were immobilized in 10% neutral buffered formalin, dehydrated in various concentrations of ethanol, embedded in paraffin, and sectioned in five-micron for hematoxylin and eosin staining. Sections were scanned using a microscopic-resolution scanner. Measurements of villus height, crypt depth, mucosal thickness were performed using NanoZoomerTM Digital Pathology software view2.

Gut microbiota: Total bacterial DNA was collected from each sample using the TIANamp Stool DNA kit. The quality of the extracted DNA was evaluated using a NanoDrop spectrophotometer. PCR amplification was then performed using primers 314f and 806r (341F: 5-CCTAYGGGRBGCASCAG-3; 806R: 5-GGACTACNNGGGTATCTAAT-3), which target the V3-V4 region of the 16S rRNA gene. The PCR mixture (30 mL) contains 15 mL of 2 ′ Phusion High-Fidelity PCR Master Mix (New England Biolabs, USA), 10 ng of DNA template, 0.6 mM of forward and reverse primers, and 2 mL of HPLC-grade water. The V3-V4 regions were amplified at 98°C for 1 min, followed by 30 cycles of 98°C for 10 s, 50°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. The PCR products were verified with 2% (wt/vol) agarose gel electrophoresis. DNA libraries were constructed using Ion Plus Fragment Library Kit (Life Technologies, USA) following the manufacturer's protocol. The next generation sequencing was performed using the Ion S5 XL platform by Novogene Bioinformatics Technology Co. Ltd. (China).

Quality control of the raw reads was performed according to steps described by Caporaso [15]. Chimeric sequences were identified and removed using UCHIME [16]. Operational Taxonomic Units (OTUs) were clustered using UPARSE (v7.0.1001) at a 97% similarity threshold using [17]. Taxonomic classifications were assigned using the SILVA SSURef database and RDP classifier via QIIME [18]. Alpha diversity estimators including Chao 1, ACE, Shannon and Simpson indexes were calculated using QIIME. Beta diversity was analyzed by principal coordinate analysis (PCoA) based on weighted UniFrac distance. Linear Discriminant Analysis of the Effect Size (LEfSe) was performed to identify significant differences among all the treatment groups [19].

Statistical analysis: Data of blood indices and intestinal morphology were examined using the general linear model of SAS. After significant interactions are detected, Duncan's multiple range test is used for multiple comparisons. Alpha diversity estimators were analyzed by using t-test between the control and the GAL-treated groups. Differences in the structure of bacterial community were determined by an Analysis of Similarity Randomization Test (ANOSIM) between the control and the GAL-treated groups. A P-value less than 0.05 indicated significant differences. LEfSe analysis was conducted by Kruskal-Wallis test, and the Logarithmic Linear Discriminant (LDA) threshold score was set to 4.0.

Results

Blood parameters: Oral administration of GAL reduced serum TNF-α in the GAL-treated groups (P=0.0001). The level of serum IL-1β was less in the GAL-H group than that in other groups (P<0.0001). The concentration of serum IL-6 was less in the GAL-M and GAL-H groups than that in the control (P<0.0001) (Figure 1). The lysozyme activity was higher in the GAL-M and GAL-H groups than that in the control (P=0.0008). The concentration of complement C3 was greatest in the GAL-H group (P<0.0001), and that of C4 was greatest in the GAL-M and GAL-H groups (P<0.0001) (Figure 2). The activities of serum SOD and GPx were higher in the GAL-M and GAL-H groups than those in the control (P=0.0488; P<0.0001). The level of serum MDA was lower in the GAL-L and GAL-H groups than that in the control (P=0.0004) (Figure 3).

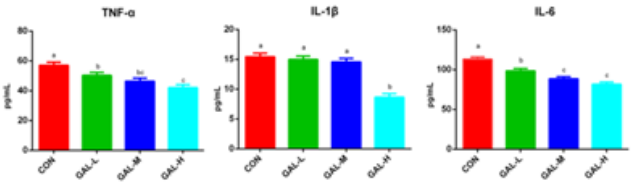

Figure 1: The concentration of serum TNF-α, IL-1β and IL-6 in the control and GAL-treated groups.

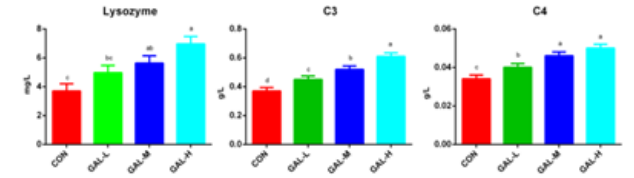

Figure 2: The activities of serum lysozyme, complement C3 and C4 in the control and GAL-treated groups.

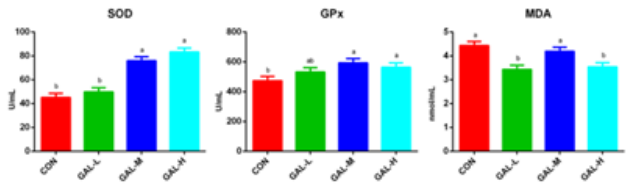

Figure 3: The concentration of serum SOD, GPx and MDA in the control and GAL-treated groups.

Intestinal morphology: Feeding GAL increased muscle thickness in the GAL-L and GAL-M groups (P=0.0028). The villus height, crypt depth, and the villus height and crypt depth ratio (V/C value) were similar among groups (Figure 4).

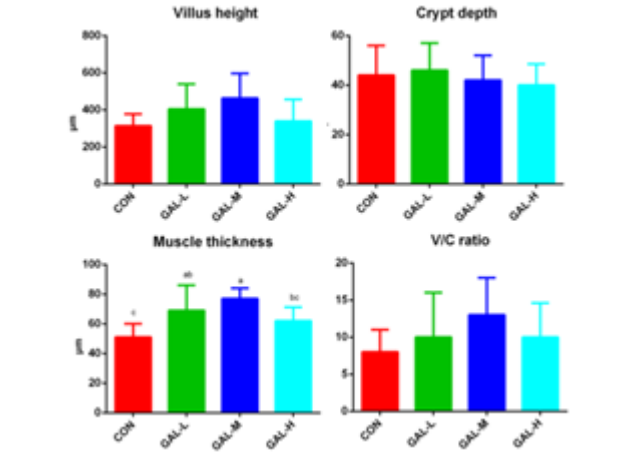

Figure 4: Intestinal morphology of fish in the control and GAL-treated groups.

Gut microbiota: A total of 2,899,472 valid DNA nucleic acid sequences were obtained from the 36 samples in this experiment. These sequences were grouped into 2,809 operational taxonomic units (OTUs) based on 97% nucleotide sequence similarity. Of all the samples, Fusobacteria were the predominant phylum (30.97%), closely followed by Firmicutes (20.36%), Proteobacteria (19.21%), Actinobacteria (13.17%), Bacteroidetes (6.53%), Spirochaetes (3.27%), Deinococcus-Thermus (0.35%), Cyanobacteria (0.29%), Acidobacteria (0.25%), Chloroflexi (0.12%), and Others (5.47%) (Figure 5).

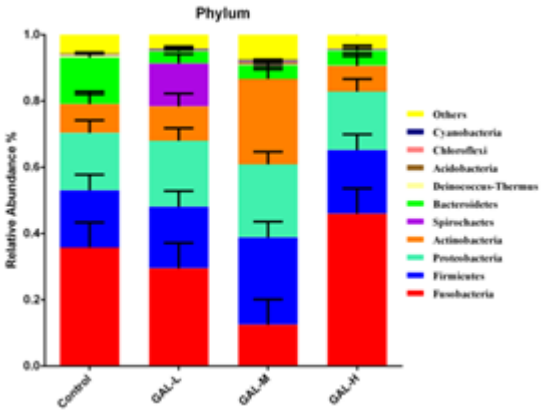

Figure 5: The 10 most abundant phyla of intestinal bacteria in the control and GAL-treated groups.

The alpha diversity was similar between each GAL-treated group and the control group. However, principal coordinate analysis (PCoA) demonstrated that the gut microbiota of the GAL-M group was different from that of the control (ANOSIM, R=0.2778, P=0.007) (Figure 6). In total, 28 taxa of varying abundance were identified as potential biomarkers through LEfSe analysis. At the phylum level, Actinobacteria was abundant in the GAL-M group, Spirochaetes was abundant in the GAL-L group, and Bacteroidetes was abundant in the control. At the genus level, Photobacterium was abundant in the GAL-H group, Propionibacterium and Citrobacter were enriched in the GAL-M group, Brevinema and Phyllobacterium were abundant in the GAL-L group, and Vibrio was abundant in the control (Figure 7).

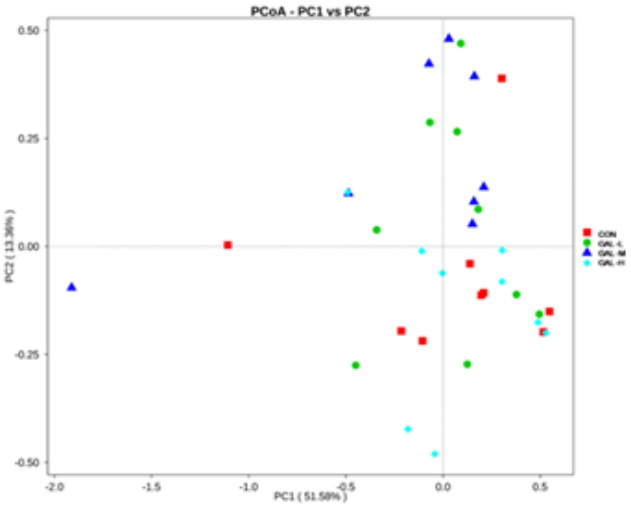

Figure 6: Principal coordinate analysis of intestinal bacterial community.

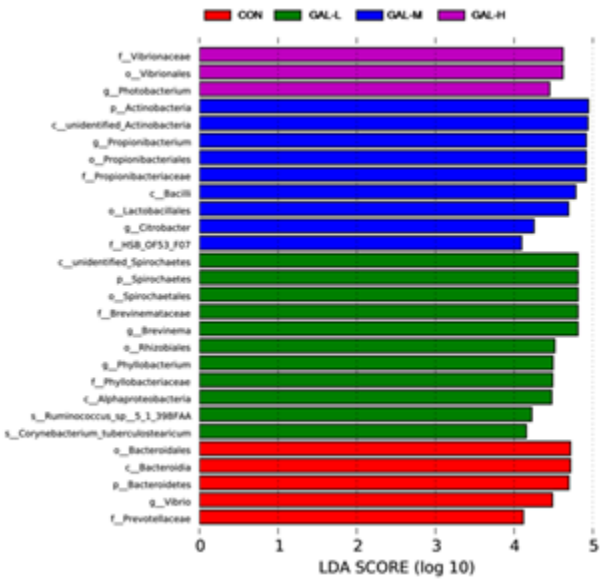

Figure 7: Linear discriminant analysis coupled with effect size (LEfSe) of gut microbiota among treatment groups.

Correlation analysis: Correlations between blood parameters and microbial abundance at the phylum and genus level are illustrated in Figure 8. Notably, the phylum Bacteroidetes was positively associated with IL-6 and MDA (P=0.0162; P=0.0056), negatively associated with lysozyme, C3, C4 and SOD (P=0.0037; P=0.0066; P=0.0284; P=0.0053), respectively. The genus Photobacterium was positively associated with lysozyme, C3, C4, and SOD (P=0.0383; P=0.0011; P=0.0140; P=0.0005), negatively associated with IL-1β and IL-6 (P=0.0006; P=0.0010), respectively.

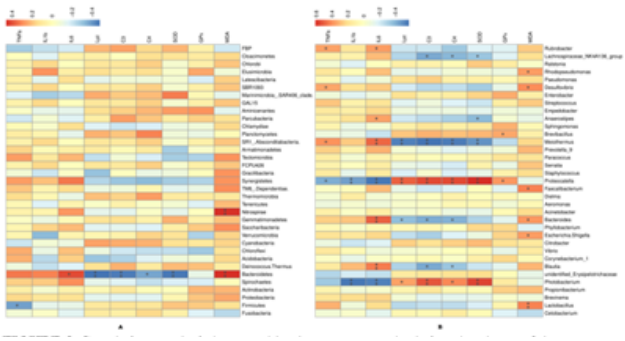

Figure 8: Correlations analysis between blood parameters and relative abundance of the 35 most abundant bacteria at (A) phylum level and (B) genus level. Blue and red represent negative and positive correlations, respectively. *, **, *** represent significant differences at $P < 0.05$, $P < 0.001$, $P < 0.0001$, respectively.

Discussion

Garlic powder has the characteristics of promoting growth [20], antibacterial [21, 22], anti-inflammatory [23, 24] and antioxidant [25, 26]. In ammonia stress experiments, the addition of garlic promoted antioxidant capacity and had a hepatoprotective effect on the liver [27]. Garlic powder increased immunoglobulins in European seabass [28], its' microencapsulated extract promoted the growth of rainbow trout and enhanced the activity of plasma proteins, albumin and lysozyme [29]. These studies show that garlic powder has been widely used to replace dietary antibiotics in animal feed [9].

Dietary supplementation with GAL has been found to induce changes in hematological parameters. Similar findings have been made previously with rainbow trout, where the addition of garlic powder to the diet increased total serum protein, albumin, and globulin levels and improved immunity [30]. Meanwhile, GAL exerted beneficial effects on non-specific immunity by enhancing lysozyme activity, phagocytic activity, and respiratory burst [30, 31]. This was further confirmed by our findings that GAL increased the level of serum lysozyme, complement C3, and complement C4. Similarly, supplementation of garlic powder in the fish diet resulted in lower levels of some substances in fish serum, such as TNF-α, IL-1β, and IL-6.

Studies have shown that feeding different concentrations of garlic powder can improve the body composition and growth of sturgeons [5]. The addition of garlic to the diet resulted in a significant increase in the body weight of rainbow trout compared to the control group, as well as an increase in SGR to varying degrees [32]. Reports shown that it can enhance obvious nutrient digestion in rainbow trout [27] and Nile tilapia [33], with improved protein efficiency and lipid efficiency. In addition, garlic powder can stimulate appetite [34, 35] and digestive enzyme secretion. We observed an improvement in intestinal histology in the experiment, which may also help to improve growth performance.

Garlic possesses broad-spectrum antimicrobial activity [21,22, 36]. Reports have shown that several aquatic pathogenic bacteria have been sensitive to garlic extracts, including A. hydrophila and Vibrio harveyi [37]. The antibacterial action of garlic relies primarily on allicin, the most important of which is thiosulfate, which has been shown to inhibit various thiol-dependent enzyme systems [38]. Moreover, some derivatives, such as Propyl Propane Thiosulfinate (PTS) and Propyl Propane Thiosulfonate (PTS-O), have antibacterial activity [39]. Dietary supplementation with PTS-O regulated the intestinal microbiota composition in growing broilers [40]. Garlic supplementation affects the bacteria in the intestinal tract and a recent study on fish showed that the addition of garlic to the diet of rainbow trout (*Oncorhynchus mykiss*) resulted in different bacterial communities in the intestinal system. We observed similar results in this study, especially when 1500 mg/kg of garlic powder was added to the feed. The intestinal microbiota differed from the control group. The biological activity of garlic powder may be related to the composition of the intestinal tract structure. We observed that Photobacterium was positively correlated with lysozyme, C3, C4, and SOD and negatively correlated with IL-1β and IL-6. This genus was abundant in the GAL-H group, so it might contribute to the enhancement of the immune response and antioxidant ability.

Conclusion

In conclusion, dietary supplementation with GAL improved non-specific immunity, antioxidative ability, and intestinal morphology.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgment

This work was supported by the General Research Project of Beijing Municipal Education Commission (KM201910020008) on "Effects of plant extracts on the intestinal flora and immune function of koi." This research was partially supported by Beijing University of Agriculture.

Statements and Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, review and editing, analysis were performed by Yibo Du, Rong Zhang and Haiyang Zhao. The first draft of the manuscript was written by Yibo Du. Funding acquisition and Project administration were performed by Yongchun Cao. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Abdel TM, Khalil RH, Diab AM, Khallaf MA, Abdel RN, et al. (2021) Dietary garlic and chitosan enhanced the antioxidant capacity, immunity, and modulated the transcription of HSP70 and Cytokine genes in Zearalenone-intoxicated European seabass. Fish Shellfish Immunology 113: 35-41.
2. Adineh H, Harsij M, Jafaryan H, Asadi M (2020) The effects of microencapsulated garlic (Allium sativum) extract on growth performance, body composition, immune response and antioxidant status of rainbow trout (Oncorhynchus mykiss) juveniles. Journal of Applied Animal Research 48: 372-378.
3. Ankri S, Mirelman D (1999) Antimicrobial properties of allicin from garlic. Microbes and Infection 1: 125-129.
4. Arreola R, Quintero FS, Ivette LRR, Octavio FGE, Pablo RGJ, et al. (2015) Immunomodulation and Anti-Inflammatory Effects of Garlic Compounds. Journal of Immunology Research 40.
5. Bakri IM, Douglas CWI (2005) Inhibitory effect of garlic extract on oral bacteria. Archives of Oral Biology 50: 645-651.
6. Buyukdeveci ME, Balcazar JL, Demirkale I, Dikel S (2018) Effects of garlic-supplemented diet on growth performance and intestinal microbiota of rainbow trout (Oncorhynchus mykiss). Aquaculture 486: 170-174.
7. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7: 335-336.
8. Chakraborty SB, Horn P, Hancz C (2013) Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. Reviews in Aquaculture 6: 1-19.
9. Citarasu T (2010) Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International 18: 403-414.
10. Diab AS, Aly SM, John G, Abde HY, Mohammed MF (2008) Effect of garlic, black seed and Biogen as immunostimulants on the growth and survival of Nile tilapia, Oreochromis niloticus (Teleostei: Cichlidae), and their response to artificial infection with Pseudomonas fluorescens. African Journal of Aquatic Science 33: 63-68.
11. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27: 2194-2200.
12. Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10: 996-998.
13. Elagib HAA, Amin EWIA, Malik HEE (2013) Effect of dietary garlic (Allium sativum) supplementation as feed additive on broiler performance and blood profile. Journal of Animal science advances 3:58-64.
14. Ellman GL (2022) Tissue Sulfhydryl Groups. Archives of Biochemistry and Biophysics 726.
15. Ernster L, Nordenbrand K (1967) [92a] Microsomal lipid peroxidation. Methods in enzymology 10: 574-580.
16. Esmaeili M, Kenari AA, Rombenso AN (2017) Effects of fish meal replacement with meat and bone meal using garlic (Allium sativum) powder on growth, feeding, digestive enzymes and apparent digestibility of nutrients and fatty acids in juvenile rainbow trout (Oncorhynchus mykiss Walbaum, 1792). Aquaculture Nutrition 23: 1225-1234.
17. Fall J, Ndong D (2007) The effect of garlic (Allium sativum) on growth and immune responses of hybrid tilapia (Oreochromis niloticus x Oreochromis aureus). National Taiwan Ocean University, Taiwan.
18. Fo T, Han X, Zhao H (1990) Research and application of garlic residue premix. Feed Industry 11: 12-13.
19. Guo JJ, Kuo CM, Chuang YC, Hong JW, Chou RL, et al. (2012) The effects of garlic-supplemented diets on antibacterial activity against Streptococcus iniae and on growth in orange-spotted grouper, Epinephelus coioides. Aquaculture 364: 33-38.
20. Harada K (1990) Attraction Activities of Spices for Oriental Weatherfish and Yellowtail. Nippon Suisan Gakkaishi 56: 2029-2033.
21. Jonkers D, Broek EVD, Dooren IV, Thijs C, Dorant E, et al. (1999) Antibacterial effect of garlic and omeprazole on Helicobacter pylori. Journal of Antimicrobial Chemotherapy 43: 837-839.
22. Lee DH, Lim SR, Han JJ, Lee SW, Ra CS, et al. (2014) Effects of Dietary Garlic Powder on Growth, Feed Utilization and Whole Body Composition Changes in Fingerling Sterlet Sturgeon, Acipenser ruthenus. Asian-Australas J Anim Sci 27: 1303-1310.
23. Lee DY, Li H, Lim HJ, Lee HJ, Jeon R, et al. (2012) Anti-Inflammatory Activity of Sulfur-Containing Compounds from Garlic. Journal of Medicinal Food 15: 992-999.
24. Lee JY, Gao Y (2012) Review of the Application of Garlic, Allium sativum, in Aquaculture. Journal of the World Aquaculture Society 43: 447-458.
25. Leontiev R, Hohaus N, Jacob C, Gruhlke MCH, Slusarenko AJ (2018) A Comparison of the Antibacterial and Antifungal Activities of Thiosulfinate Analogues of Allicin. Scientific Reports 8.
26. Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry 47: 469-474.
27. Mohebbi A, Nematollahi A, Dorcheh EE, Asad FG (2011) Influence of dietary garlic (A llium sativum) on the antioxidative status of rainbow trout (O ncorhynchus mykiss). Aquaculture Research 43: 1184-1193.
28. Mustapha MK (2017) Comparative assessment of the water quality of four types of aquaculture ponds under different culture systems. Advanced Research in Life Sciences 1: 104-110.
29. Naeiji N, Shahsavani D, Baghshani H (2013) Effect of dietary garlic supplementation on lipid peroxidation and protein oxidation biomarkers of tissues as well as some serum biochemical parameters in common carp Cyprinus carpio. Fisheries Science 79: 699-705.
30. Nerurkar LS (1981) 67 – Lysozyme. Methods for studying mononuclear phagocytes 667-683.

31. Nya EJ, Austin B (2009) Use of garlic, Allium sativum, to control Aeromonas hydrophila infection in rainbow trout, Oncorhynchus mykiss (Walbaum). Journal of Fish Diseases 32: 963-970.

32. Peinado MJ, Ruiz R, Echavarri A, Aranda OI, Rubio LA (2013) Garlic derivative PTS-O modulates intestinal microbiota composition and improves digestibility in growing broiler chickens. Animal Feed Science and Technology 181: 87-92.

33. Pula HJ, Trenzado CE, Garcia MS, Fallola C, Sanz A (2018) Effects of different culture systems on growth, immune status, and other physiological parameters of tench (Tinca tinca). Aquaculture 485: 101-110.

34. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41: 590-596.

35. Ruiz R, García MP, Lara A, Rubio LA (2010) Garlic derivatives (PTS and PTS-O) differently affect the ecology of swine faecal microbiota in vitro. Veterinary Microbiology 144: 110-117.

36. Sasmal D, Babu CS, Abraham TJ (2005) Effect of garlic (Allium sativum) extract on the growth and disease resistance of Carassius auratus (Linnaeus, 1758). Indian J Fish 52: 207-214.

37. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, et al. (2011) Metagenomic biomarker discovery and explanation. Genome Biology 12.

38. Shalaby AM, Khattab YA, Abdel RAM (2006) Effects of Garlic (Alliumsativum) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (Oreochromis niloticus). Journal of Venomous Animals and Toxins including Tropical Diseases 12: 172-201.

39. Talpur AD, Ikhwanuddin M (2012) Dietary effects of garlic (Allium sativum) on haemato-immunological parameters, survival, growth, and disease resistance against Vibrio harveyi infection in Asian sea bass, Lates calcarifer (Bloch). Aquaculture 364: 6-12.

40. Vibertigc GC (1990) The immunotqurbidimetric of componement 3 and 4. clin Chemistry 36: 1109-1174.

HERALD

Advances In Industrial Biotechnology | ISSN: 2639-5665

Advances In Microbiology Research | ISSN: 2689-694X

Archives Of Surgery And Surgical Education | ISSN: 2689-3126

Archives Of Urology

Archives Of Zoological Studies | ISSN: 2640-7779

Current Trends Medical And Biological Engineering

International Journal Of Case Reports And Therapeutic Studies | ISSN: 2689-310X

Journal Of Addiction & Addictive Disorders | ISSN: 2578-7276

Journal Of Agronomy & Agricultural Science | ISSN: 2689-8292

Journal Of AIDS Clinical Research & STDs | ISSN: 2572-7370

Journal Of Alcoholism Drug Abuse & Substance Dependence | ISSN: 2572-9594

Journal Of Allergy Disorders & Therapy | ISSN: 2470-749X

Journal Of Alternative Complementary & Integrative Medicine | ISSN: 2470-7562

Journal Of Alzheimers & Neurodegenerative Diseases | ISSN: 2572-9608

Journal Of Anesthesia & Clinical Care | ISSN: 2378-8879

Journal Of Angiology & Vascular Surgery | ISSN: 2572-7397

Journal Of Animal Research & Veterinary Science | ISSN: 2639-3751

Journal Of Aquaculture & Fisheries | ISSN: 2576-5523

Journal Of Atmospheric & Earth Sciences | ISSN: 2689-8780

Journal Of Biotech Research & Biochemistry

Journal Of Brain & Neuroscience Research

Journal Of Cancer Biology & Treatment | ISSN: 2470-7546

Journal Of Cardiology Study & Research | ISSN: 2640-768X

Journal Of Cell Biology & Cell Metabolism | ISSN: 2381-1943

Journal Of Clinical Dermatology & Therapy | ISSN: 2378-8771

Journal Of Clinical Immunology & Immunotherapy | ISSN: 2378-8844

Journal Of Clinical Studies & Medical Case Reports | ISSN: 2378-8801

Journal Of Community Medicine & Public Health Care | ISSN: 2381-1978

Journal Of Cytology & Tissue Biology | ISSN: 2378-9107

Journal Of Dairy Research & Technology | ISSN: 2688-9315

Journal Of Dentistry Oral Health & Cosmesis | ISSN: 2473-6783

Journal Of Diabetes & Metabolic Disorders | ISSN: 2381-201X

Journal Of Emergency Medicine Trauma & Surgical Care | ISSN: 2378-8798

Journal Of Environmental Science Current Research | ISSN: 2643-5020

Journal Of Food Science & Nutrition | ISSN: 2470-1076

Journal Of Forensic Legal & Investigative Sciences | ISSN: 2473-733X

Journal Of Gastroenterology & Hepatology Research | ISSN: 2574-2566

Journal Of Genetics & Genomic Sciences | ISSN: 2574-2485

Journal Of Gerontology & Geriatric Medicine | ISSN: 2381-8662

Journal Of Hematology Blood Transfusion & Disorders | ISSN: 2572-2999

Journal Of Hospice & Palliative Medical Care

Journal Of Human Endocrinology | ISSN: 2572-9640

Journal Of Infectious & Non Infectious Diseases | ISSN: 2381-8654

Journal Of Internal Medicine & Primary Healthcare | ISSN: 2574-2493

Journal Of Light & Laser Current Trends

Journal Of Medicine Study & Research | ISSN: 2639-5657

Journal Of Modern Chemical Sciences

Journal Of Nanotechnology Nanomedicine & Nanobiotechnology | ISSN: 2381-2044

Journal Of Neonatology & Clinical Pediatrics | ISSN: 2378-878X

Journal Of Nephrology & Renal Therapy | ISSN: 2473-7313

Journal Of Non Invasive Vascular Investigation | ISSN: 2572-7400

Journal Of Nuclear Medicine Radiology & Radiation Therapy | ISSN: 2572-7419

Journal Of Obesity & Weight Loss | ISSN: 2473-7372

Journal Of Ophthalmology & Clinical Research | ISSN: 2378-8887

Journal Of Orthopedic Research & Physiotherapy | ISSN: 2381-2052

Journal Of Otolaryngology Head & Neck Surgery | ISSN: 2573-010X

Journal Of Pathology Clinical & Medical Research

Journal Of Pharmacology Pharmaceutics & Pharmacovigilance | ISSN: 2639-5649

Journal Of Physical Medicine Rehabilitation & Disabilities | ISSN: 2381-8670

Journal Of Plant Science Current Research | ISSN: 2639-3743

Journal Of Practical & Professional Nursing | ISSN: 2639-5681

Journal Of Protein Research & Bioinformatics

Journal Of Psychiatry Depression & Anxiety | ISSN: 2573-0150

Journal Of Pulmonary Medicine & Respiratory Research | ISSN: 2573-0177

Journal Of Reproductive Medicine Gynaecology & Obstetrics | ISSN: 2574-2574

Journal Of Stem Cells Research Development & Therapy | ISSN: 2381-2060

Journal Of Surgery Current Trends & Innovations | ISSN: 2578-7284

Journal Of Toxicology Current Research | ISSN: 2639-3735

Journal Of Translational Science And Research

Journal Of Vaccines Research & Vaccination | ISSN: 2573-0193

Journal Of Virology & Antivirals

Sports Medicine And Injury Care Journal | ISSN: 2689-8829

Trends In Anatomy & Physiology | ISSN: 2640-7752