

Original Research Articles

Effects of PS-MPs on growth, immunity, antioxidant capacity and amino acid metabolism in common carp (*Cyprinus carpio*)

Pengcheng Li¹, Weijun Chen¹, Deshan Chen¹, Pengfei Xie¹, Lei Han¹, Feng Yang¹, Ping Sun^{1a}¹ Henan University of Science and Technology

Keywords: PS-MPs, common carp, growth, immunity, antioxidant, amino acid metabolism

<https://doi.org/10.46989/001c.133894>

Israeli Journal of Aquaculture - Bamidgeh

Vol. 77, Issue 2, 2025

To explore the toxic effects of polystyrene microplastics (PS-MPs) on common carp (*Cyprinus carpio*), 0 µg/L, 50 µg/L, 100 µg/L, and 200 µg/L PS-MPs were designated as the control group (CK) and low, medium, and high concentration groups, respectively. Each group had 3 replicates and was exposed to PS-MPs for 15 days. The results showed that, compared with the control group, the weight gain rate and hepatopancreas somatic indices were significantly decreased in 100 and 200 µg/L PS-MPs groups, the visceral index was significantly decreased in 50 and 100 µg/L PS-MPs groups. In comparison to CK, experimental groups (50-200 µg/L PS-MPs) significantly decreased activity levels of acid phosphatase, alkaline phosphatase, and peroxidase while significantly increased activity levels of lysozyme, catalase, and superoxide dismutase. In addition, the malondialdehyde contents of the experimental carp increased significantly in 50 and 100 µg/L PS-MPs groups compared to CK. For hepatic amino acid contents, methionine, threonine, leucine, and isoleucine contents of the hepatopancreas of carp in PS-MPs groups were significantly lower than the control group. Correlation analysis showed that amino acids in the liver closely associated with fish growth, immune, and antioxidant-related indicators. In conclusion, PS-MPs above 50 µg/L in the water body could significantly affect normal growth, weaken the activity of hepatopancreatic immunity-related enzymes and antioxidant capacity, and disturb the balance of amino acid metabolism in common carp. The findings provide valuable insights into optimizing feeding strategies, contribute to more sustainable aquaculture practices, and support the development of policies in aquatic ecosystems.

INTRODUCTION

In the context of modern industrialization, plastics are widely used in all aspects of daily life because they are inexpensive, lightweight, malleable, and chemically stable. Today, the world produces more than 300 million tons of plastics annually, of which more than 20% enter the environment as pollutants and are subsequently degraded into microplastic particles by light, heat, wind, acid, and salt, gradually accumulating in the environment. Due to their microscopic size and high mobility, microplastics (MPs, < 5mm) threaten ecosystems and biological health.¹⁻³

Researchers have found MPs within specimens of more than 1,300 species of fish, birds, and mammals, including in human tissues and organs.⁴ Studies have confirmed that MPs can directly affect animal growth, reproduction, and metabolism through various toxicity mechanisms, including histopathological damage, intestinal flora disruption, oxidative stress, DNA damage, genotoxicity, reproductive

toxicity, and neurotoxicity.⁵ Moreover, MPs can indirectly affect metabolic and synthetic processes such as glucose metabolism, lipid metabolism, and energy metabolism, which further affect the growth and development of organisms.⁶ In addition, toxic substances such as persistent organic pollutants (POPs) and heavy metals adsorbed in MPs or contained in the MPs themselves can be released into the aquatic environment continuously, with toxic effects on aquatic organisms.⁷

In recent years, MPs have been widely reported to be found in marine, estuarine, river, lake, reservoir, and other aquatic environments. MPs from land-based sources and the impact of aquaculture production activities⁸ will inevitably threaten the fisheries environment. Studying the pollution characteristics of MPs and their effects on aquatic animals and aquaculture systems is of great significance to the scientific response to MPs and to guarantee the green development of aquaculture. In current studies, research on MPs has mostly focused on the toxic effects on model or-

a Corresponding author: Ping Sun, E-mail: pingsunny@msn.com

ganisms, while aquaculture fish are less frequently studied. Common carp (*Cyprinus carpio*) is one of the world's most widely bred freshwater fish, with nearly 1.9 million tons produced in 2020 and accounting for more than 50% of inland aquaculture production as of 2022.⁹ Amino acids play a crucial role in the growth and development of fish, and there are no reports on the effect of MPs on amino acid metabolism in carp culture. This experiment was conducted to investigate the effects of polystyrene microplastics (PS-MPs) on the growth performance, immunity and antioxidant ability, and amino acid metabolism of carp, to provide some references for the healthy aquaculture of carp and environmental protection.

MATERIALS AND METHODS

EXPERIMENTAL MATERIALS

Common carp used in the experiment were purchased from a fishery firm in Zhengzhou City, Henan Province. They were around 8 months old and uniform in size [body mass (24.8 ± 1.9) g, body length (10.1 ± 0.5) cm]. They were kept in acrylic pools (60 cm × 40 cm × 50 cm). 5 μm mono-dispersed PS-MPs were purchased from Jiangsu Zhichuan Science and Technology Co, Ltd.

EXPERIMENTAL DESIGN

During this experiment, the carp were kept in the aquaculture laboratory of Henan University of Science and Technology. The rearing conditions were kept stable, with 60 L of culture water, oxygen solubility ≥ 5 mg/L, water pH 7.5–8.0, water temperature $26 \pm 1^\circ\text{C}$, and daily light time of 14 h. After 2 weeks of acclimatization, 180 healthy and active carp were selected as the experimental fish, which were randomly divided into four concentrations (0, 50, 100, and 200 μg/L) of PS-MPs groups, three replicates were set up for each group, and 15 experimental fish were used in each parallel unit, accumulating 12 pools. The PS-MPs exposure experiment lasted for 15 days.

EXPERIMENTATION PROCEDURE

The experimental fish were fed twice a day (9:00 and 16:00) amounting to 3% of the carp's body weight. The activity of carp was observed daily, and food residues and feces were removed in time. The water was changed once every 24 h, and the amount of water changed was one-fourth (15 L) of the culture water, directly add the corresponding amount of MPs suspension, and then disperse it by supplement water, aiming to make it evenly distributed as far as possible. During the experimental process, the fish in the pool were weighed every 7 days to adjust the subsequent feeding amount. No mortality was observed throughout the experiment. The experimental process complied with the requirements of the Experimental Animal Management and Ethical Welfare Committee of Henan University of Science and Technology.

SAMPLING

After 15 days of PS-MPs exposure, the carp were fasted for 24 h and then sampled for analysis. For sampling, carp were put into the water containing 100 mg/L MS-222 anesthetic in advance, followed by rapid dissection, removal of hepatopancreas tissues into prepared tissue tubes, placed in liquid nitrogen for quick-freezing, and then frozen in -80°C refrigerator for the detection of alkaline phosphatase (AKP), acid phosphatase (ACP), lysozyme (LZM), superoxide dismutase (SOD), malondialdehyde (MDA), peroxidase (POD), catalase (CAT), total protein (TP) and amino acid content.

MEASUREMENT OF PARAMETERS RELATED TO CARP GROWTH PERFORMANCE

Survival rate (SR), Weight gain rate (WGR), feed conversion ratio (FCR), hepatopancreas somatic indices (HSI), and visceral index (VSI) of carp were calculated according to the following formulae.

$$\text{SR (\%)} = \text{Final fish count} / \text{Initial fish count} \times 100\%$$

$$\text{WGR (\%)} = (W_t - W_0) / W_0 \times 100\%$$

$$\text{FCR} = \text{Feed consumption} / (W_t - W_0)$$

$$\text{HSI (\%)} = (W_h / W_t) \times 100\%$$

$$\text{VSI (\%)} = (W_v / W_t) \times 100\%$$

W_0 and W_t are the initial and final body weight of fish (g) at the beginning and end of the experiment; feed consumption refers to the total amount of feed (dry weight); W_h is the weight of fish's hepatopancreas (g); W_v is the weight of fish's viscera (g).

DETECTION OF ANTIOXIDANT AND IMMUNE-RELATED PARAMETERS

MDA contents and AKP, ACP, LZM, SOD, POD and CAT activity levels were determined by using relevant test kits produced by Nanjing Jiancheng Institute of Biological Engineering, and the specific operation was referred to the instruction manual of the relevant kits.¹⁰

DETERMINATION OF AMINO ACID CONTENT IN HEPATOPANCREAS

The amino acid content in carp hepatopancreas was determined using a gas chromatography-tandem mass spectrometry (GC-MS/MS) metabolomics technical method, and the steps included sample collection, sample pretreatment, metabolite derivatization, and detection. An appropriate amount of sample was taken into an EP tube, and a pre-cooled methanol-acetonitrile mixture was added, shaken for 1 min, and sonicated for 10 min, after which the sample was allowed to stand at -20°C . After standing, the samples were centrifuged for 10 min, and 100 μL of the supernatant from the centrifugation was removed and transferred to a wide lined tube to be swished dry and subjected to a two-step derivatization process. Thereafter, 80 μL methicillin hydrochloride pyridine solution (concentration 15 mg/mL) was added to a glass vial. After that, vortex shaking was performed for 2-min and the oxidation reaction was carried

out in a shaking incubator at 37°C for 60-min. After the oxidation reaction, the samples were removed, and 80 µL of BSTFA derivatization reagent and 20 µL of hexane were added. Vortex shaking was continued for 2-min and the reaction was carried out at 70°C for 60-min. Finally, the samples were removed and left at room temperature for 30-min, followed by GC-MS metabolomics analysis, and the concentrations of the target substances in the test samples were calculated based on the standard curves, which were converted to obtain the actual content of the target substances in the samples.

STATISTICS ANALYSIS

The data obtained from the experiments were expressed as mean ± standard error of the mean (SEM), and all data were subjected to One-way ANOVA using SPSS 27.0 software. GraphPad Prism 9 software (GraphPad Software Inc., La Jolla, CA, USA) and OriginPro 2024 (OriginLab Inc., USA) were used for graphing, and comparisons between groups were made using the Least Significant Difference (LSD) method, and t-test was employed for comparisons with $P < 0.05$ indicating a significant difference between groups.

RESULTS

EFFECTS OF PS-MPS ON GROWTH INDEXES OF COMMON CARP

After 15 days of PS-MPs exposure, the carp did not die during the whole process. Compared with the control group, carp in 100 and 200 µg/L PS-MPs groups demonstrated lower WGR and HSI and higher FCR ($P < 0.05$). Carp exposed to 50 µg/L PS-MPs did not affect WGR, FCR, and HSI, but significantly reduced VSI. A significantly lower VSI was also found in carp exposed to 100 µg/L PS-MPs compared to CK ($P < 0.05$). There was no significant change in VSI between 200 µg/L group and CK (Fig. 1).

EFFECTS OF PS-MPS ON THE ACTIVITY OF IMMUNE-RELATED ENZYMES OF COMMON CARP

Compared with the control group, the activities of ACP and AKP in the experimental groups were significantly lower ($P < 0.05$), and there was no significant difference between the experimental groups, while the content of LZM was significantly higher ($P < 0.05$) and was highest in 100 µg/L groups, with a significant difference within the groups ($P < 0.05$) (Fig. 2).

EFFECTS OF PS-MPS ON ANTIOXIDANT INDICES IN THE HEPATOPANCREAS OF COMMON CARP

Compared with the control group, the MDA content of the carp in 50 and 100 µg/L PS-MPs groups was significantly higher ($P < 0.05$), while significantly lower ($P < 0.05$) in the 200 µg/L PS-MPs group; the POD activity of carp in the experimental groups was significantly lower ($P < 0.05$); the CAT activity of carp in the experimental group increased significantly ($P < 0.05$) with increasing PS-MPs concentra-

tion, and there was no significant difference between the 100 and 200 µg/L PS-MPs groups; the SOD activity of carp in the experimental group were all significantly higher ($P < 0.05$), and the SOD, POD, and CAT activity were all basically concentration dependent (Fig. 3).

EFFECTS OF PS-MPS ON AMINO ACID METABOLISM IN THE HEPATOPANCREAS OF COMMON CARP

The absolute quantification of the metabolites of the samples was counted, and the results after analyzing the amino acid species with significant differences are shown in Fig. 4. From the results of the experiment, it can be seen that after exposure to PS-MPs, the content of methionine, threonine, leucine and isoleucine in the experimental groups appeared to be significantly reduced compared with that of the control group ($P < 0.05$); the phenylalanine content were significantly decreased ($P < 0.05$) in the 100 µg/L PS-MPs group, and no significant changes occurred in 50 and 200 µg/L PS-MPs groups; the lysine content did not change significantly in 50 and 100 µg/L PS-MPs groups, and a significant increase ($P < 0.05$) was observed in the 200 µg/L PS-MPs group.

CORRELATION ANALYSIS OF AMINO ACID CONTENT WITH GROWTH, IMMUNITY AND ANTIOXIDANT INDEXES

Pearson correlation analysis was used to analyze the relationship between carp amino acid content with growth, immunity, and antioxidant indexes (Fig. 5). The results showed that WGR was negatively related to Lys ($P < 0.05$). FCR positively linked with Lys ($P < 0.05$). HSI and VSI had a significant and positive correlation with Met, Thr, Leu, Ile, and Phe ($P < 0.05$). ACP, AKP and POD positively correlated with the contents of Met, Thr, Leu and Ile ($P < 0.01$); MDA negatively and significantly correlated with the contents of Lys and Phe ($P < 0.05$).

DISCUSSION

EFFECTS OF PS-MPS ON THE GROWTH AND AMINO ACID METABOLISM OF COMMON CARP

WGR is an important indicator of fish growth, and its decrease may mean a disturbance in the feeding, digestive, or absorptive processes in fish.¹¹ In 100 and 200 µg/L PS-MPs groups, the WGR was significantly reduced, indicating that PS-MPs (> 100 µg/L) reduced fish growth. The phenomenon can be explained from two aspects. Firstly, the accumulation of PS-MPs particles in the digestive tract of carp, occupied the space of the food, resulting in the inability of carp to obtain sufficient nutrients.¹² Secondly, PS-MPs may also affect the activity of digestive enzymes and the structure of intestinal microbial communities in carp,¹³ which in turn may affect their nutrient absorption and utilization. Moreover, the increase of FCR is also a phenomenon of concern. The FCR reflects the efficiency of feed utilization in the growth process of fish. In this study, the FCR was significantly higher in 100 and 200 µg/L PS-MPs groups, indi-

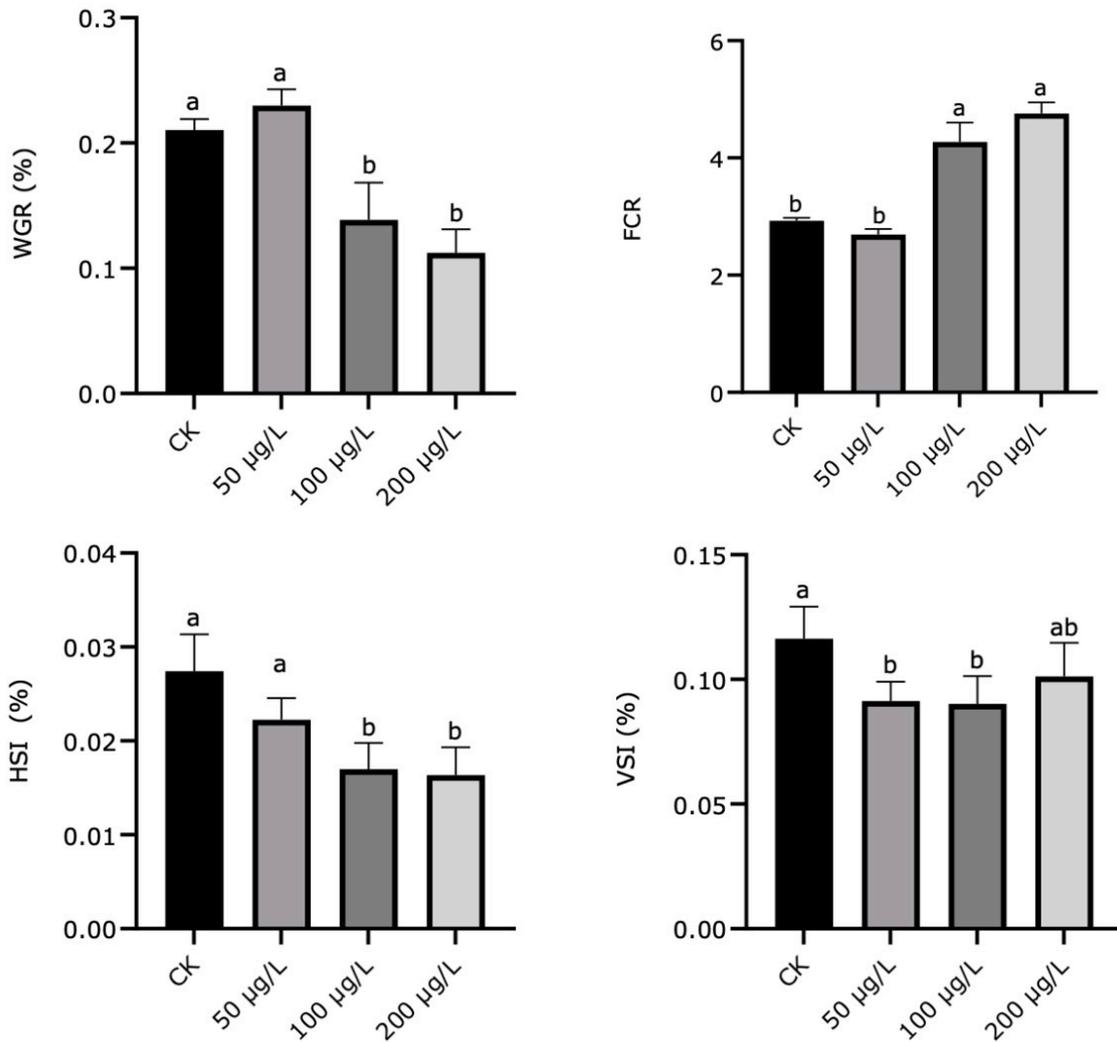


Fig. 1. Effects of PS-MPs on growth indexes of common carp

Note: different lowercase letters represent significant differences between groups ($P < 0.05$), the same below.

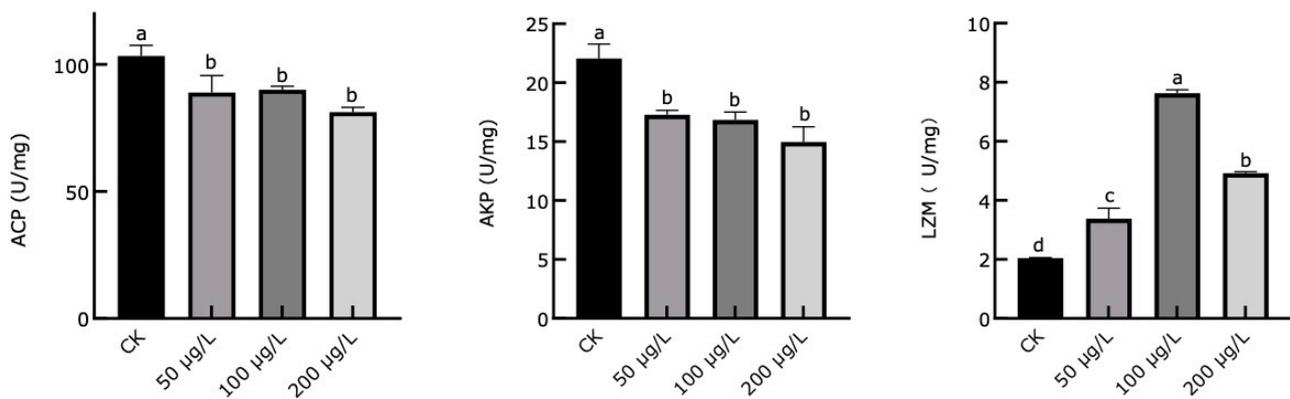


Fig. 2. Effects of PS-MPs on immune-related enzymes activity of common carp

cating that carp need to consume more feed to obtain the same weight gain. This may be due to the presence of PS-MPs reducing the appetite of carp or affecting their ability to digest and absorb the feed, which in turn interferes with energy metabolism.⁶ In addition, changes in HSI and VSI

provided clues about the effects of PS-MPs on the internal organs of carp. The decrease in HSI may mean that the functions of hepatopancreas are affected, such as detoxification and metabolism may be decreased.¹⁴ A decrease in VSI indicates that the weight or function of internal organs

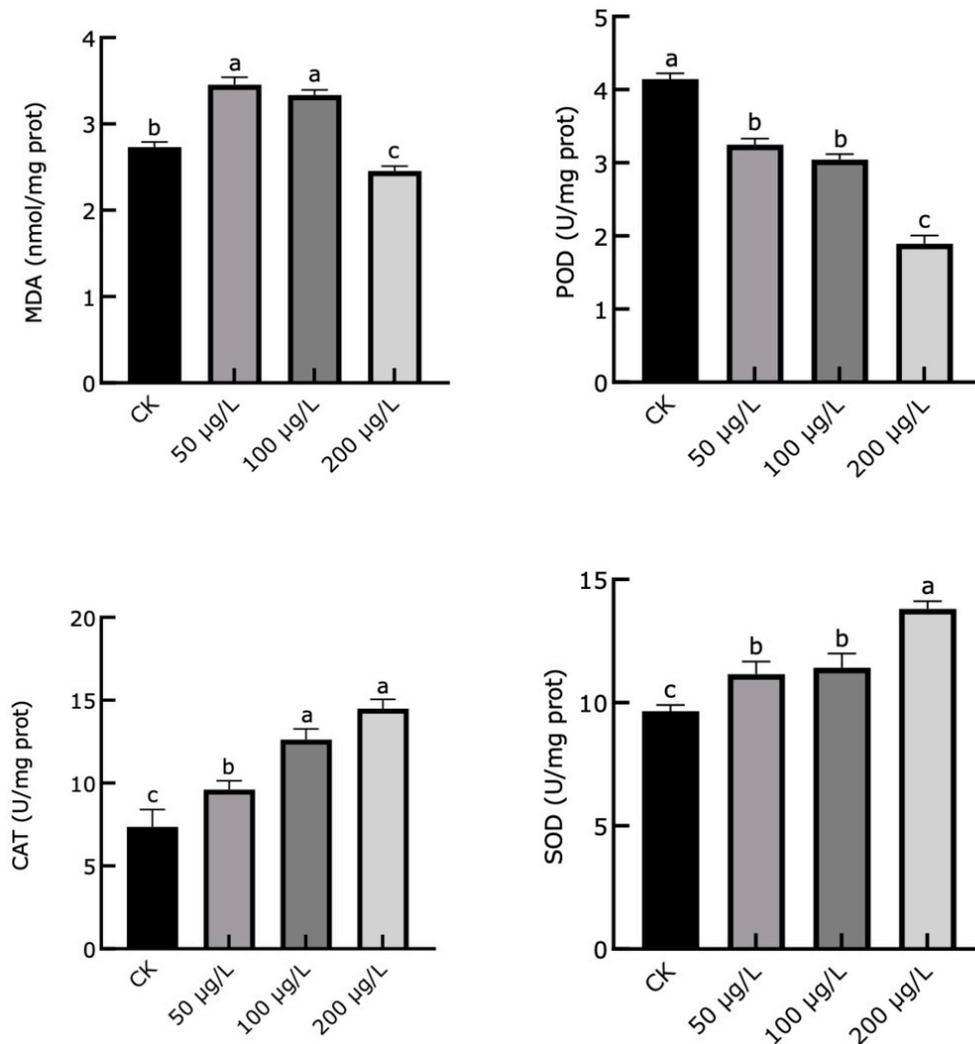


Fig. 3. Effects of PS-MPs on antioxidant indices in common carp

is affected. These changes may further affect the health status and viability of the carp. It is noteworthy that there was no significant change in the WGR and FCR of carp in the 50 µg/L PS-MPs group, which may be related to the concentration effect of PS-MPs. However, this does not mean that there is no potential risk to carp from low concentrations of PS-MPs, as long-term exposure to low concentrations of PS-MPs may still have chronic effects on common carp.

Previous studies in aquatic animals have suggested that fish growth performance is closely associated with amino acids.¹⁵ The liver is an important organ in carrying out amino acid metabolism. Thus, we measured the amino acid content of the liver and analyzed the relationship between the amino acid content and the growth indexes of carp. The effects of the feed intake of aquatic animals and its deficiency will lead to a decline in growth performance, which has been reported in various fish species, such as carp, catfish (*Silurus asotus*), and flounder (*Pleuronectiformes*), and with the increase of Thr level, the feed intake of these fishes are reduced (Liu. et al., 2024; Yu-Wen et al.¹⁶). Leu is essential for maintaining various physiological functions in the body, and appropriate levels of Leu were found to promote growth and protein synthesis in largemouth bass (*Mi-*

cropterus salmoides).¹⁷ Met, Thr, Leu, Ile, and Phe are essential amino acids, and the sole source of these essential amino acids was from the diets. A decrease in the liver's amino acid content may indicate a potential deficiency in the intake of these amino acids. The study on rainbow trout (*Oncorhynchus mykiss*) has also indicated that dietary Leu, Ile, and Thr levels positively correlated with Leu, Ile, and Thr contents in the liver.¹⁸ In this study, the contents of Met, Thr, Leu, and Ile in carp showed a significant decrease after PS-MPs exposure, which reflected a deficiency in intake of these amino acids and would affect the normal amino acid metabolism and protein synthesis process of the carp. It can adversely affect the normal growth and development of carp, which was reflected in the significant changes in the WGR, FCR, HSI, and VSI, thus hindering the growth of the carp. Correlation analysis also showed a positive correlation between HSI and VSI with the content of Met, Thr, Leu, Ile, and Phe. Similarly, the study on golden pompano (*Trachinotus ovatus*)¹⁹ reported that VSI and HSI elevated with increasing Ile (13.2-18.2 g/kg dry feed). In addition, PS-MPs also affected Phe metabolism. Phe plays an important role in growth and development, as well as in the production of neurotransmitters, hormones and proteins,

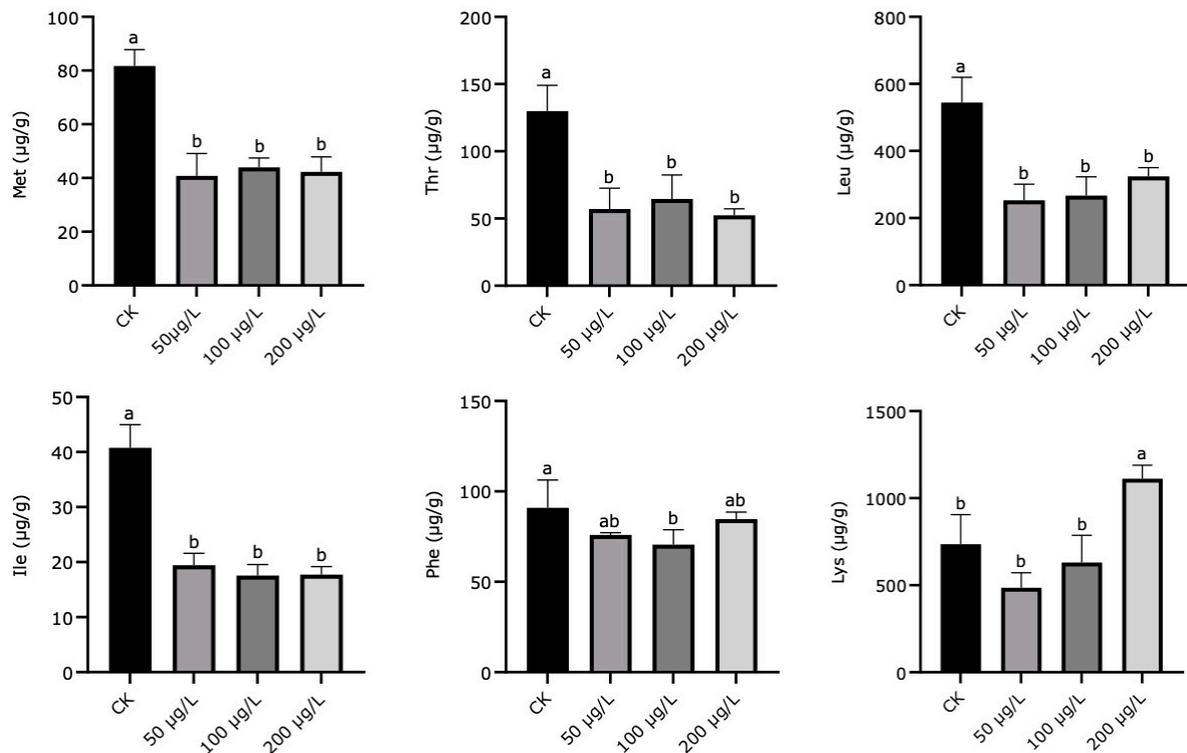


Fig. 4. Effects of PS-MPs on amino acid content of common carp hepatopancreas

which in turn are involved in metabolic processes such as glucose metabolism and fat metabolism in the body. Lack of Phe can lead to mental confusion, depression, memory loss, and low energy levels.²⁰

EFFECTS OF PS-MPS ON IMMUNITY, ANTIOXIDANT, AND AMINO ACID METABOLISM IN COMMON CARP

ACP and AKP, as key enzymes closely related to organismal immunity, are involved in various metabolic processes in the body and are important biological indicators for assessing the damage caused by exogenous substances to fish.²¹ ACP enables cells to recognize foreign substances and accelerates phagocytosis and degradation of foreign substances by phagocytes. AKP is related to nutrient absorption and transport and can act as an important detoxification system in the body. In this study, the experimental groups' ACP and AKP activities showed a significant decreasing trend with the increase of PS-MPs concentration after 15 days of exposure. This suggests that PS-MPs inhibited the activities of ACP and AKP in the carp, and the decrease in their activities may induce an inflammatory response, which in turn may affect the immune function of the carp organism. Similar results to the present experiment were obtained in an exposure experiment to PVC-MPs in carp, where corresponding changes in the activity of ACP, AKP, LZM, and the expression levels of a range of immune-related genes were observed.²² LZM is a key defense molecule of the innate immune system, produced in vivo mainly by neutrophils and macrophages, which can mediate the defense mechanism against exogenous pathogen infection through the complement system and macrophages, and its

activity can be affected by a variety of factors such as temperature, acidity and alkalinity, and heavy metal ions.²³ LZM content in the carp of experimental groups was significantly higher, indicating that the immune response of the organism plays a role under the stress of PS-MPs. These results indicate that PS-MPs can significantly disrupt the homeostasis of relevant immune-enzymes in the carp, produce immunotoxicity, and lead to the development of immune stress.²⁴

MDA is a product of lipid peroxidation and can be used as an indicator of the degree of oxidative damage in the body. SOD, CAT and POD are common antioxidant enzymes in the body and have the ability to scavenge free radicals and protect the cells from oxidative damage.²⁵ The MDA content of experimental groups was significantly different compared with the control group, indicating that PS-MPs stress disturbed the reactive oxygen species balance in the body of carp and caused oxidative stress in the carp. MDA content in 50 and 100 µg/L PS-MPs groups was significantly higher, but the MDA content of the 200 µg/L PS-MPs group was significantly lower, suggesting that the carp's immune system played a role in inhibiting PS-MPs-induced oxidative stress, perhaps by inducing an increase in the activity of antioxidant enzymes, which is in line with the results of the relevant studies on the effect of PS-MPs on crucian carp (*Carassius auratus*).²⁶ The results of this experiment showed that both SOD and CAT activity increased significantly with the increase of PS-MPs concentration, and the enzyme activity was also at its highest value at the highest concentration of PS-MPs, but the MDA content was significantly reduced, which may suggest that the activities of these two antioxidant enzymes in the carp were activated

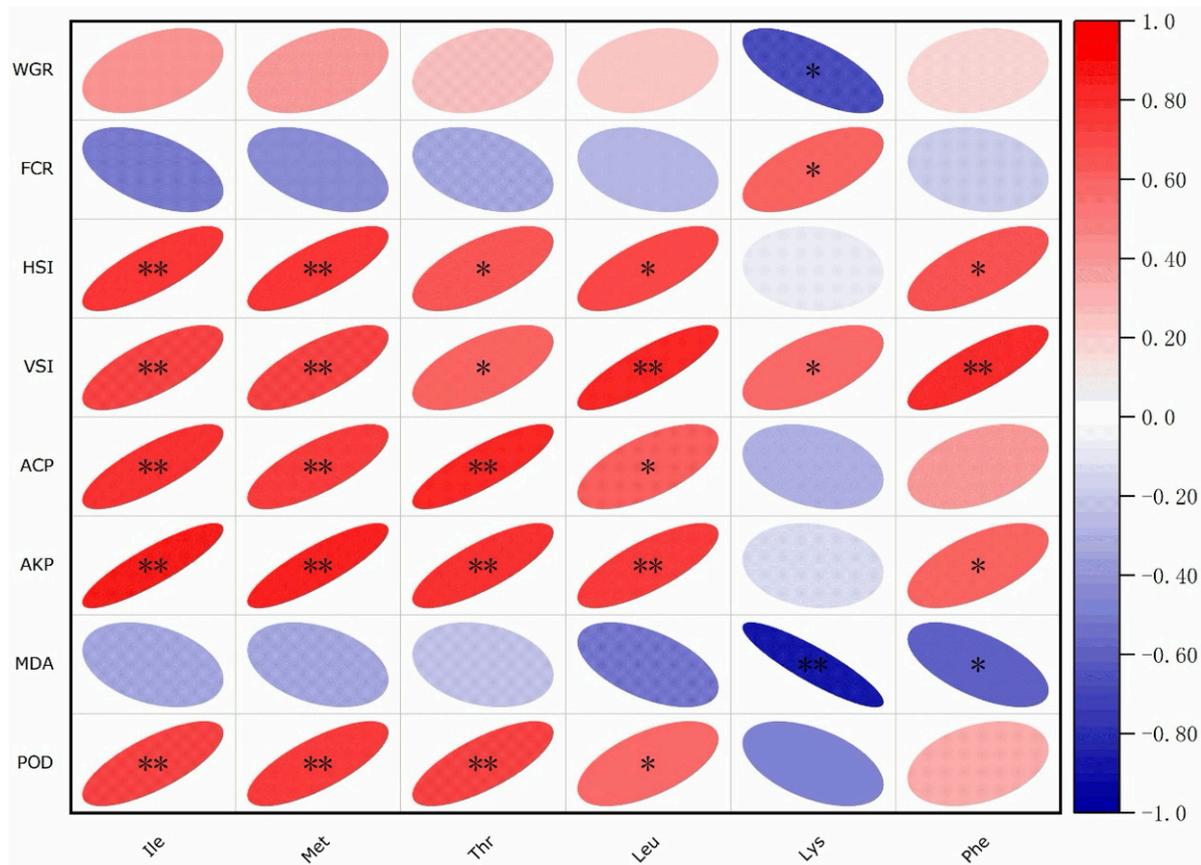


Fig. 5. Correlation analysis of amino acid content with growth, immunity and antioxidant indexes of common carp

Note: * $p < 0.05$ ** $p < 0.01$, Red indicates a positive correlation and blue indicates a negative correlation

after being stressed by PS-MPs, which reduced the content of MDA, and thus came to cope with the challenge of oxidative stress. Similar results were obtained in a study where PVC-MPs were applied to common carp.²⁷ POD is an important enzymatic antioxidant that scavenges hydrogen peroxide radicals and other toxic molecules from cells and protects them from oxidative damage. In the results of this experiment, the POD activity in the carp of experimental groups was significantly reduced compared with the control group, and the lowest POD activity was found in the high-concentration group, indicating that the increase in the concentration of PS-MPs exacerbated the oxidative stress response in the carp, and the POD activity was not enough to scavenge the increase in the amount of reactive oxygen species in the carp, which led to the decrease in its activity.

In terms of amino acid metabolism, Met, Leu, Ile, and Thr as essential amino acids in fish, play an important role in enhancing antioxidant and immune function. Thr is an important nutritional fortifier in the animal body and has the effect of improving immune functions.²⁸ Ile can significantly enhance the physical barrier function of fish gills and intestines and improve the phagocytosis and bactericidal efficacy of phagocytes, thus enhancing the non-specific immunity of fish.²⁹ In this study, correlation analysis showed a positive correlation between ACP, AKP, and POD with the content of Met, Thr, Leu, indicating that PS-MPs induced oxidative stress and immune response in the body of carp

and affected the related amino acid metabolism process, and interfered with the synthesis or catabolism of some amino acids, affecting the balance of amino acid metabolism in carp, which was associated with a variety of pathological and physiological development processes. Similarly, the experiment found that high levels of Leu could enhance the activities of intestinal amylase, promote hepatic and pancreatic glycolysis and gluconeogenesis, as well as hepatic lipid catabolism, and reduce inflammation when Ile is deficient or in excess, cells may be at risk of oxidative damage.³⁰

Our results align with previous research on marine species, which also reports adverse effects such as reduced growth, oxidative stress, and immune suppression due to MPs exposure. However, our study highlights that freshwater species may exhibit distinct physiological responses, potentially due to differences in habitat conditions and species-specific sensitivities. These findings underscore the broader relevance of MPs impacts across aquatic ecosystems and emphasize the need for tailored mitigation strategies in both marine and freshwater aquaculture systems. To address MPs exposure in aquaculture, our findings suggest that integrating advanced filtration technologies, such as membrane filtration or biofilters, could effectively reduce microplastic contamination in water sources. Additionally, modifying feed practices, such as using microplastic-free feed or incorporating natural binders to reduce PS-MP up-

take, could further mitigate risks. These strategies, informed by our results, could enhance the sustainability and resilience of aquaculture systems globally.

CONCLUSIONS

In summary, PS-MPs significantly affect the common carp's growth indicators, immune and antioxidant enzyme activities, and amino acid metabolism. These changes may negatively impact their health condition and nutritional value, consequently reducing the economic value generated by common carp farming. Therefore, this study emphasizes that the potential threat of PS-MPs pollution to fish health and consumer safety needs to be urgently addressed. The control of PS-MPs levels in the aquatic environment during common carp culture is a key direction for future research. To mitigate these risks, it is recommended that aquaculture industries adopt stricter monitoring and filtration systems to reduce microplastic contamination in water sources. Additionally, policymakers should consider implementing regulations to limit plastic waste and promote sustainable practices to safeguard aquatic ecosystems and the economic viability of fish farming.

.....

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation Youth Foundation. (Grant numbers: 32202952).

AUTHORS' CONTRIBUTION

Conceptualization: Pengcheng Li (Equal), Ping Sun (Equal). Methodology: Pengcheng Li (Equal), Deshan Chen (Equal), Pengfei Xie (Equal). Formal Analysis: Pengcheng Li (Equal), Deshan Chen (Equal), Pengfei Xie (Equal). Investigation: Pengcheng Li (Equal), Deshan Chen (Equal), Pengfei Xie (Equal). Writing – original draft: Pengcheng Li (Lead). Writing – review & editing: Weijun Chen (Equal), Lei Han (Equal), Feng Yang (Equal), Ping Sun (Equal). Funding acquisition: Weijun Chen (Equal), Ping Sun (Equal). Resources: Ping Sun (Lead). Supervision: Ping Sun (Lead).

COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

This experiment complies with the requirements of the Experimental Animal Management and Ethical Welfare Committee of Henan University of Science and Technology.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

Submitted: January 17, 2025 CST. Accepted: February 18, 2025 CST. Published: May 20, 2025 CST.



This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CCBY-4.0). View this license's legal deed at <http://creativecommons.org/licenses/by/4.0> and legal code at <http://creativecommons.org/licenses/by/4.0/legalcode> for more information.

REFERENCES

1. Degnan T. Waste-plastic processing provides global challenges and opportunities. *Mrs Bulletin*. 2019;44(6):436-437. doi:[10.1557/mrs.2019.133](https://doi.org/10.1557/mrs.2019.133)
2. Gao P. Chasing “Emerging” Contaminants: An Endless Journey toward Environmental Health. *Environmental Science & Technology*. 2024;58(4):1790-1792. doi:[10.1021/acs.est.3c10630](https://doi.org/10.1021/acs.est.3c10630)
3. Núñez-Delgado A, Zhang Z, Bontempi E, Coccia M, Race M, Zhou YY. Editorial on the Topic “New Research on Detection and Removal of Emerging Pollutants.” *Materials*. 2023;16(2):2. doi:[10.3390/ma16020725](https://doi.org/10.3390/ma16020725)
4. Leslie HA, van Velzen MJM, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH. Discovery and quantification of plastic particle pollution in human blood. *Environment International*. 2022;163:8. doi:[10.1016/j.envint.2022.107199](https://doi.org/10.1016/j.envint.2022.107199)
5. He TW, Qu Y, Yang XH, et al. Research progress on the cellular toxicity caused by microplastics and nanoplastics. *Journal of Applied Toxicology*. 2023;43(11):1576-1593. doi:[10.1002/jat.4449](https://doi.org/10.1002/jat.4449)
6. Zhang YK, Yang BK, Zhang CN, Xu SX, Sun P. Effects of polystyrene microplastics acute exposure in the liver of swordtail fish (*Xiphophorus helleri*) revealed by LC-MS metabolomics. *Science of The Total Environment*. 2022;850:9. doi:[10.1016/j.scitotenv.2022.157772](https://doi.org/10.1016/j.scitotenv.2022.157772)
7. Ting P, Hao L, Fang Y, et al. Review of microplastics in lakes: sources, distribution characteristics, and environmental effects. *Carbon Research*. Published online 2023. doi:[10.1007/s44246-023-00057-1](https://doi.org/10.1007/s44246-023-00057-1)
8. Lin L, Chen CC, Zhu XS, Pan K, Xu XR. Risk of aquaculture-derived microplastics in aquaculture areas: An overlooked issue or a non-issue? *Frontiers in Marine Science*. 2022;9:6. doi:[10.3389/fmars.2022.923471](https://doi.org/10.3389/fmars.2022.923471)
9. Khanjani MH, Sharifinia M, Hajirezaee S. Biofloc: A sustainable alternative for improving the production of farmed cyprinid species. *Aquaculture Reports*. 2023;33:22. doi:[10.1016/j.aqrep.2023.101748](https://doi.org/10.1016/j.aqrep.2023.101748)
10. Xie YX, Yang XM, Kaneko G, et al. Effects of different stocking densities and feeding frequencies on growth, physiological and biochemical indexes, and intestinal microflora of largemouth bass (*Micropterus salmoides*) under land-based round po. *Aquaculture*. Published online 2024:580. doi:[10.1016/j.aquaculture.2023.740385](https://doi.org/10.1016/j.aquaculture.2023.740385)
11. Xie L, Li P, Zhu XS, et al. Hepatotoxic of polystyrene microplastics in aged mice: Focus on the role of gastrointestinal transformation and AMPK/FoxO pathway. *Science of The Total Environment*. 2024;917:16. doi:[10.1016/j.scitotenv.2024.170471](https://doi.org/10.1016/j.scitotenv.2024.170471)
12. Kankiliç GB, Koraltan I, Erkmen B, et al. Size-selective microplastic uptake by freshwater organisms: Fish, mussel, and zooplankton. *Environmental Pollution*. 2023;336:11. doi:[10.1016/j.envpol.2023.122445](https://doi.org/10.1016/j.envpol.2023.122445)
13. Wang FH, Zhang QR, Cui J, et al. Polystyrene microplastics induce endoplasmic reticulum stress, apoptosis and inflammation by disrupting the gut microbiota in carp intestines. *Environmental Pollution*. 2023;323:12. doi:[10.1016/j.envpol.2023.121233](https://doi.org/10.1016/j.envpol.2023.121233)
14. Ping X, Pengcheng L, Xiaoshan Z, et al. Hepatotoxic of polystyrene microplastics in aged mice: Focus on the role of gastrointestinal transformation and AMPK/FoxO pathway. *Science of The Total Environment*. Published online 2024. doi:[10.1016/j.scitotenv.2024.170471](https://doi.org/10.1016/j.scitotenv.2024.170471)
15. Xing SJ, Liang XF, Zhang XR, et al. Essential amino acid requirements of fish and crustaceans, a meta-analysis. *Reviews in Aquaculture*. 2024;16(3):1069-1086. doi:[10.1111/raq.12886](https://doi.org/10.1111/raq.12886)
16. Yu-Wen D, Wei-Dan J, Yang L, et al. Threonine deficiency decreased intestinal immunity and aggravated inflammation associated withNF-κBtarget of rapamycinsignalling pathways in juvenile grass carp (*Ctenopharyngodon idella*) after infection with *Aeromonas hydrophila*. *British Journal of Nutrition*. Published online 2017. doi:[10.1017/s0007114517001830](https://doi.org/10.1017/s0007114517001830)
17. Xu C, Huang XP, Guan JF, et al. Effects of dietary leucine and valine levels on growth performance, glycolipid metabolism and immune response in Tilapia GIFT *Oreochromis niloticus*. *Fish & Shellfish Immunology*. 2022;121:395-403. doi:[10.1016/j.fsi.2022.01.028](https://doi.org/10.1016/j.fsi.2022.01.028)
18. Takeshi Y, Tatsuya U, Toshio A. The influence of dietary protein and fat levels on tissue free amino acid levels of fingerling rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. Published online 2000. doi:[10.1016/s0044-8486\(99\)00277-x](https://doi.org/10.1016/s0044-8486(99)00277-x)

19. Huang Z, Lin HZ, Peng JS, et al. Effects of Dietary Isoleucine Levels on the Growth Performance, Feed Utilization, and Serum Biochemical Indices of Juvenile Golden Pompano, *Trachinotus ovatus*. *Israeli Journal of Aquaculture-Bamidgeh*. 2015;67:9.
20. Li FX, Sun Y, Sun XX, Hu Y, Wu YL. Self-assembled amphiphilic vesicles based on chiral H8-BINOL to enantioselective recognition of d- and l-phenylalanine. *Dyes and Pigments*. 2024;222:9. doi:[10.1016/j.dyepig.2023.111859](https://doi.org/10.1016/j.dyepig.2023.111859)
21. Chi C, Giri SS, Jun JW, et al. Effects of algal toxin okadaic acid on the non-specific immune and antioxidant response of bay scallop (*Argopecten irradians*). *Fish & Shellfish Immunology*. 2017;65:111-117. doi:[10.1016/j.fsi.2017.03.031](https://doi.org/10.1016/j.fsi.2017.03.031)
22. Liu XY, Liang CA, Zhou M, Chang ZJ, Li L. Exposure of *Cyprinus carpio* var. larvae to PVC microplastics reveals significant immunological alterations and irreversible histological organ damage. *Ecotoxicology and Environmental Safety*. 2023;249:8. doi:[10.1016/j.ecoenv.2022.114377](https://doi.org/10.1016/j.ecoenv.2022.114377)
23. Song Q, Xiao Y, Xiao ZH, et al. Lysozymes in Fish. *Journal of Agricultural and Food Chemistry*. 2021;69(50):15039-15051. doi:[10.1021/acs.jafc.1c06676](https://doi.org/10.1021/acs.jafc.1c06676)
24. Hirt N, Body-Malapel M. Immunotoxicity and intestinal effects of nano- and microplastics: a review of the literature. *Particle and Fibre Toxicology*. 2020;17(1):22. doi:[10.1186/s12989-020-00387-7](https://doi.org/10.1186/s12989-020-00387-7)
25. Ding P, Xiang CD, Li XT, et al. Photoaged microplastics induce neurotoxicity via oxidative stress and abnormal neurotransmission in zebrafish larvae (*Danio rerio*). *Science of The Total Environment*. 2023;881:9. doi:[10.1016/j.scitotenv.2023.163480](https://doi.org/10.1016/j.scitotenv.2023.163480)
26. Yang H, Xiong HR, Mi KH, Xue W, Wei WZ, Zhang YY. Toxicity comparison of nano-sized and micron-sized microplastics to Goldfish *Carassius auratus* Larvae. *Journal of Hazardous Materials*. 2020;388:11. doi:[10.1016/j.jhazmat.2020.122058](https://doi.org/10.1016/j.jhazmat.2020.122058)
27. Chen X, Wang J, Xie YJ, et al. Physiological response and oxidative stress of grass carp (*Ctenopharyngodon idellus*) under single and combined toxicity of polystyrene microplastics and cadmium. *Ecotoxicology and Environmental Safety*. 2022;245:9. doi:[10.1016/j.ecoenv.2022.114080](https://doi.org/10.1016/j.ecoenv.2022.114080)
28. Wu S, Liu XZ, Cheng L, et al. Protective Mechanism of Leucine and Isoleucine against H₂O₂-Induced Oxidative Damage in Bovine Mammary Epithelial Cells. *Oxidative Medicine and Cellular Longevity*. 2022;2022:22. doi:[10.1155/2022/4013575](https://doi.org/10.1155/2022/4013575)
29. Gu CS, Mao XB, Chen DW, Yu B, Yang Q. Isoleucine Plays an Important Role for Maintaining Immune Function. *Current Protein & Peptide Science*. 2019;20(7):644-651. doi:[10.2174/1389203720666190305163135](https://doi.org/10.2174/1389203720666190305163135)
30. Mao XB, Gu CS, Ren M, et al. L-Isoleucine Administration Alleviates Rotavirus Infection and Immune Response in the Weaned Piglet Model. *Frontiers in Immunology*. 2018;9:17. doi:[10.3389/fimmu.2018.01654](https://doi.org/10.3389/fimmu.2018.01654)